JOURNAL

OF

HELMINTHOLOGY

Edited by
R. T. LEIPER, M.D., D.Sc., F.R.S.,
Professor of Helminthology in the University of London.

Vol. XIII, 1935.

Institute of Agricultural Parasitology,
Winches Farm,
Hatfield Road,
St. Albans, Herts,
England.

CONTENTS OF VOLUME XIII.

No. 1 (February, 1935).

PAGE

1. CLAPHAM, P. A. On the Experimental Transmission of *Syngamus trachea* from Starlings to Chickens 1

2. CLAPHAM, P. A. The Treatment of Gapeworm Disease ... 3

3. CLAPHAM, P. A. On Nodules Occasioned by Gapeworm in Pheasants 9

4. OLDHAM, J. N. On the Occurrence of *Rhabditis coarctata* (Nematoda) on Caterpillars in the Marquesas Islands 13

5. GOODEY, T. On *Cylindrogaster curzii* n. sp., a saprophagous Nematode 19

6. ANDREWS, M. N. The Complement Fixation Reaction in *Schistosoma japonicum* with Cercarial Antigen prepared from *Oncomelania hupensis* 25

7. TETLEY, J. H. Ecological Studies on *Nematodirus* species in Sheep in Manawatu district, New Zealand 41

No. 2 (May, 1935).

1. TRIFFITT, M. J. On cyst-like bodies, resembling cysts of *Heterodera schachtii* of common occurrence in British Soils 59

2. BUCKLEY, J. J. C. Some Observations on *Necator suillus* Ackert and Payne, 1922 67

PAGE

3. CARROLL, J. and MCMAHON, E. Potato Eelworm (*Heterodera schachtii*) Investigations 77

4. GOODEY, T. Observations on a Field Plot Experiment with *Anguillulina dipsaci* on Potatoes 91

5. LAPAGE, G. The Second Ecdysis of the Infective Larvae of Certain Trichostrongylidae in Solutions of Sodium Sulphide and of Organic Compounds containing Sulphur 103

6. LAPAGE, G. The Behaviour of sterilised exsheathed infective Trichostrongylid larvae in sterile media resembling their environment in Ovine hosts 115

7. EDWARDS, E. E. On *Heterodera schachtii*, with special reference to the Oat race in Britain 129

No. 3 (July, 1935).

1. CLAPHAM, P. A. Some Helminth Parasites from Partridges and other English Birds 139

2. TRIFFITT, M. J. On the origin of strains of *Heterodera schachtii* occurring in Britain, with special reference to the Beet-strain 149

3. PETERS, B. G. The Vinegar Eelworm in Tan Liquor ... 159

4. OLDHAM, J. N. Further Observations on the Incidence of Parasitism of Flea Beetles by the Nematode, *Howardula phyllotretae* 163

5. GOODEY, T. *Aphelenchoides hodsoni* n. sp., a nematode affecting Narcissus bulbs and leaves 167

6. GOODEY, T. Observations on a Nematode Disease of Yams 173

PAGE

No. 4 (December, 1935).

1. HURST, R. H. and TRIFFITT, M. J. Experiments on the Control of "Potato-Sickness" by the addition of Certain Chemicals to Soil infected with *Heterodera schachtii* 191

2. HURST, R. H. and TRIFFITT, M. J. Calcium Cyanamide and other Artificial Fertilisers in the Treatment of Soil infected with *Heterodera schachtii* 201

3. TRIFFITT, M. J. and HURST, R. H. On the Thermal Death-point of *Heterodera schachtii* 219

4. GOODEY, T. *Brevibucca saprophaga* gen. et sp. nov., a nematode from a rotting lily bulb-scale 223

Index 229

Journal of Helminthology, Vol. XIII, *No.* 1, *Feb.*, 1935, *pp.* 1–2.

On the Experimental Transmission of *Syngamus trachea* from Starlings to Chickens.

By PHYLLIS A. CLAPHAM, Ph.D.

(*Lawrence Research Student of the Royal Society.*)
(*From the Institute of Agricultural Parasitology, St. Albans.*)

In a previous paper (1934) it was shown that the life-cycle of *S. trachea* proceeds best when an intermediate host is involved. By means of *Eisenia foetida*, an annulate worm, it was possible to induce heavy and certain infestations of gapeworm in chickens with material obtained from the partridge, pheasant, rook and chicken. Other earthworms were shown to be possible intermediate hosts but this was found to be the best and most successful.

During the past summer (1934), some gapeworms from starlings came into the laboratory and the transmission of this material, to chickens, was effected using *Eisenia foetida* as intermediate host. In the first experiment 2 small gapeworms were obtained from a young starling. The eggs were cultured and fed to 3 *E. foetida*, which were then fed to a chicken aged 5 days. After 11 days it was killed and 3 pairs of gapeworms were recovered from the top of the trachea.

A few weeks later 11 pairs of gapeworms from starlings were cultivated and the infective eggs fed to *E. foetida*. These were fed to 4 chickens. One of these died 36 hours later and showed severe lesions in the lungs, from which adolescent gapeworms were obtained by dissection. The other 3 survived the pulmonary stage and later developed " gapes." At post-mortem they contained 4, 6 and 6 pairs of gapeworms respectively.

On a third occasion 3 pairs of starling gapes were available from which 2 chicks were infected. Both died of the disease. One contained 22 pairs and the other 16 pairs of gapeworms. They were very small but mature. These also lay at the top of the trachea.

Though the numbers of experimental animals were of necessity small, owing to lack of much material, yet with 100 per cent. infections, there seems to be no doubt of the infectivity of this strain to chickens. This is contrary to the view of Taylor who in 1928, on the results of attempts to

transmit starling gapes to chickens, not using an intermediate host says, " . . . it must be concluded that this strain of *Syngamus trachea* of starlings did not take kindly to its new host at the first passage, and under natural conditions would not be so likely to cause trouble among chicks as would the introduction of infection from the chicken host." These present results show quite conclusively that, using the indirect method of infection, the starling strain of gapeworms is very infective to chickens and that starlings are therefore of considerable importance in the spread of the diseases.

CLAPHAM, P. A., 1934. " Experimental Studies on the Transmission of Gapeworm (*Syngamus trachea*) by Earthworms." *Proc. roy. Soc. B.*, CXV 18–29. (*W.L.* 16900).

TAYLOR, E. L., 1928. " *Syngamus trachea* from the Starling, transferred to the Chicken, and some Physiological Variations Observed." *Ann. Trop. Med. Parasit.*, XXII (3), 307–318. (*W.L.* 1063).

Journal of Helminthology, Vol. XIII, *No.* 1, *Feb.*, 1925, *pp.* 3–8.

The Treatment of Gapeworm Disease.

By Phyllis A. Clapham, B.Sc., Ph.D.

(*Lawrence Research Student of the Royal Society*)

(*From the Institute of Agricultural Parasitology, St. Albans, London School of Hygiene and Tropical Medicine*)

In a note in the British Medical Journal for February 3rd, 1934, there is mention of the anthelmintic action of garlic oil. One paragraph says that good results have been obtained in the treatment of threadworm in children while a second is more specific and quotes the case of a woman who was cured of *Ascaris lumbricoides* in 36 hours. She passed a male, 13¾ inches long, a female and a bunch of newly hatched young.

Dr. R. T. Leiper brought this to my notice and suggested that as garlic oil is excreted by the lungs it might be effective in the treatment of gapeworm disease. Messrs. Allen and Hanburys Ltd. very kindly supplied me with natural oil and with the synthetic product, allyl sulphide. I am much obliged to them for this favour.

Many attempts have been made in the past to find a cure for gapeworm disease but these have met with but little success as, being sidetracked in the trachea, the worms are somewhat inaccessible. Treatments by drugs via the gut have never met with enough success to warrant their use. Fumigation and inhalation have been tried: the principle being that the inhaled fumes would either irritate the worms until they released their hold or would stupify them so that, in either case, they could be coughed up by the host. Intratracheal injection of a dilute (5–8 per cent.) solution of salicylic acid by means of a small syringe has been recommended but the most popular method of treatment seems to have been the mechanical removal of the worms by means of twisted horse hairs or of partially stripped feathers. This method is not entirely successful and has the disadvantage of being tedious to the operator and irritating to the chicken. In many cases of disease, however, it would appear that the custom has been to let the chicks die. Up to the present prophylaxis has been the more useful line of work.

With garlic oil the present writer has had some considerable success. Three methods of treatment have been used. The first was the anointing of the feathers of the head and neck so that the birds were compelled to inhale the fumes. No success was realised by this method and by reason of its odour, it had the great disadvantage of being very unpleasant to both human beings and birds. The second method was to mix the oil with the food or water but this was not successful either. One minim of oil per bird was mixed with the day's ration of food but the birds refused to eat, even though this food was left there in front of them without change for some time until it had become stale. Even when the birds were compelled to eat a quantity, by means of forcible feeding, yet cure did not occur. There are two possible causes of this. The birds probably did not get a sufficient concentration of oil passing through the lungs and up the trachea to kill the parasites, or it may be that the presence of food may alter the absorption or reaction of the drug.

That the birds found this concentrated oil very unpleasant was obvious by the fact that when mixed with the food or water, they refused to eat or drink it and it was impossible to make them take it by themselves. It was necessary therefore to administer it by means of a fine pipette which could be inserted well back into the throat. This led to the third method of treatment, which proved highly satisfactory.

Pipettes were drawn out finely so that the oil could be administered in minims. When smaller doses were required, a solution was made up in linseed oil. The usual percentage of oil in these solutions was either 50% or $33\frac{1}{3}$%.

The birds used in these experiments had been artificially infected with the parasite using the intermediate host *Eisenia foetida*. The method in general use was to divide the birds into two groups, one of which remained untreated as a control. In the other group the faeces were examined before and after treatment and after the oil had been administered search was made in the cage for any worms that might have been expelled. When the treatment was over, the birds were killed and the tracheae in the two groups compared. As will be seen later it was almost impossible to discover the number of worms expelled by the chickens by searching in the cage as the drug caused them to rupture. Comparisons with the control group were not satisfactory either as it was not possible to estimate the degree of infection of the intermediate host. Hence in later experiments this control group was dropped and more reliance was placed on the

disappearance of clinical symptoms and of eggs in the faeces. Furthermore when the birds were killed within a few hours after the last treatment with the drug, it was often possible to see a small inflamed papilla, with a petechial haemorrhage, indicating the site of adhesion of a worm.

Altogether three sets of experiments were carried out, involving about 50 birds. The dosage ranged from $\frac{1}{3}$ minim to 3 minims of pure oil. It may be remarked here that no difference was observed between the actions of the natural and the synthetic products. Hence the synthetic product, allyl sulphide, is preferable as this seems to have the slightly less pungent odour and is considerably cheaper to buy.

The chicks used were between the ages of 13 and 22 days and the most successful treatment was as follows :—$\frac{1}{3}$ minim of the drug in linseed oil solution was given three times a day orally by pipette. Plenty of water was allowed all day and food was placed before them about 5·30 p.m. and left until morning. A small feed was given about 2 p.m. The times and method of dosing is given in a table, together with a representative sample of results.

It was found that 2 minims per day divided into three doses was the limit that an infected chick could stand : even then there was a mortality of over 6 per cent. This, given in a single dose, killed practically all the infected birds though healthy birds were not quite so severely affected. Less than $\frac{1}{2}$ minim of oil per day was not satisfactory as an anthelmintic.

The effect of the garlic oil is very marked and is quickly seen. During the first day heavily infected birds cease gaping and only the sneezing is seen. This latter symptom gradually moderates and about three days after treatment, has subsided and the birds seem cured. Three days was usually enough for the birds to cough up the parasite which had been released by the oil.

The action of the drug is interesting. The parasites are markedly affected and by some reaction the body wall is weakened and ruptured allowing the genitalia to escape and be exposed. The coughing of the host caused the fine tubes to break and to be gradually brought up. Because of this reaction it was impossible to recover gapeworms from the floor of the cage during treatment, though portions were sometimes observed. That the action of garlic oil is very powerful was seen in the fact also that all the eggs recovered were sterilised. On several occasions, attempts were made to culture them but no development ever occurred. The contents were dark and opaque and within a day or two, decomposition

with the deposition of oil granules was apparent. It was rare to find eggs that had escaped this action of the oil. This seems to the writer to be an observation of importance, as on occasions, a number of parasites were not dislodged as a result of treatment but the eggs were always non-viable and therefore would no longer be a source of danger to other birds.

It was, however, realised that individual treatment of chickens would probably prove to be a tedious business and not always practical during an epidemic. For this reason attempts were made to administer the oil in food or water. These have already been described.

Summarising these results we may say that garlic oil has a very marked effect on *S. trachea*. It causes the worm to rupture, thus killing the parasites when the birds may cough them up. The eggs which the gapeworms contain are sterilised even though the worms may be retained within the body for a longer or shorter period after treatment.

Carbon tetrachloride was another drug used against *S. trachea*. In a letter to Nature 1931, p. 585, Atkins mentions that he has used this drug against gapes successfully. In a private letter to me he kindly gave me his technique and his results. The treatment seems to be quite useless, however, for chicks up to 14 days old.

The CCl_4 was administered by means of a pipette as a solution in thick medicinal paraffin. Two solutions—containing respectively 5 and 10 per cent. of the drug were used. It was given orally. Treatment was never hurried : it usually took about 5 minutes to give the dose to the chicken. By this means the substance was made to hang about the throat and the chick almost gargled during this time, so that the substance reached the trachea by two routes—by inhalation during the actual administration and later by excretion from the lungs after absorption from the blood stream.

Some success is to be recorded as a result of treatment with this substance. It was found extremely useful for chickens that were suffering severely and in the last stages of the disease. In no case, however, was a complete cure effected but considerable relief was often apparent. The severe symptoms disappear rapidly and though living parasites were always found at post-mortem yet they were never in sufficient numbers to cause distress to the bird.

A 5 per cent. solution was found to be the most effective and the most satisfactory dose was 1 cc. twice a day. The first was usually given about 9.30 a.m. and the second at 5.30 p.m. Considerable alleviation was

Chick.	Age in days	Treatment				Died	Remarks
		Substance	Date	Time	Quantity		
18	21	Natural oil	14.3.34	11 a.m.	minims 1	Killed 27.3.34	Gaping ceased but eggs still passed in faeces. 1 pair gapes retained.
6	21	Natural oil	14.3.34	11 a.m.	2	14.3.34 2 p.m.	6 pairs of gapes—dead and ruptured.
10	21	Natural oil	14.3.34	11 a.m.	3	14.3.34 1.30 p.m.	5 pairs of gapes—dead and ruptured.
14	21	Synthetic oil	14.3.34	11 a.m.	1	Killed 16.3.34	5 pairs of gapes—dead and ruptured.
12	21	Synthetic oil	14.3.34	11 a.m.	2	Killed 15.3.34	4 pairs of gapes—as above
13	21	Synthetic oil	14.3.34	11 a.m.	3	14.3.34 2 p.m.	21 pairs of gapes, all much broken up.
15	19	Natural oil	27.7.34 28.7.34	11 a.m. 4 p.m. 9 a.m. 1 p.m. 4 p.m.	$\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$	Killed 2.8.34	All symptoms disappeared by 1.8.34. No eggs found in the faeces and no worms were in trachea.
3	13	Synthetic oil	21.6.34 22.6.34	11 a.m. 4 p.m. 9 a.m. 1 p.m. 4 p.m.	$\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$	Killed 4.7.34	Few infertile eggs in the faeces. 1 pair of gapes in trachea and some fragments of female genitalia, containing infertile eggs. 5 papillae in trachea, indicating position of other gapes.
7	13	Synthetic oil	21.6.34 22.6.34	11 a.m. 4 p.m. 9 a.m. 1 p.m. 4 p.m.	$\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$ $\frac{1}{3}$	Killed 4.7.34	Eggs in faeces. 1 pair of gapes, brown but still alive. All eggs were infertile.
19	13	Natural oil	21.6.34 22.6.34	11 a.m. 4 p.m. 9 a.m. 1 p.m. 4 p.m.	$\frac{2}{3}$ $\frac{2}{3}$ $\frac{2}{3}$ $\frac{2}{3}$ $\frac{2}{3}$	Killed 27.6.34	All symptoms had ceased. Some broken genital tubes in trachea and 1 male. 7 papillae on wall.
26	13	Synthetic oil	as above	as above		Killed 26.6.34	No gaping but some sneezing remained. A mass of genital tubes was lodged near top of trachea in mucus. All eggs were sterile.

Table 1. Indicating the results obtained from the use of garlic oil in a representative sample of birds.

observed after a single day's treatment and the birds continued to improve after another day's treatment though fertile eggs still appeared in the faeces. Further treatment never effected a cure. In two cases, very heavily affected birds were available for experiment. Both these birds were very sick and showed every appearance of an early death. They were 15 days old at the time: the infection being of 6 days duration. They were each given 1 cc. of the 5 per cent. solution 4 times a day, at 9.30 a.m., noon, 3.30 p.m. and 6 p.m. In the evening they were much stronger and could stand instead of lying collapsed on the floor of the cage. Next day a considerable improvement was obvious. They were much stronger and were feeding though they were still gaping. Treatment was discontinued for 36 hours owing to the toxic action of the drug on the liver. Then they were given another dose which was the last. The faeces were examined for 4 days after this and fertile eggs were found continuously. They were killed and at post-mortem one showed a single pair of gapes and the other 3 pairs in the trachea. The mucosa of the trachea was inflamed and from the presence of 5 small papillae one can assume that 5 pairs of gapeworms had been removed in each bird by means of this treatment. When the birds were killed, they were still sneezing though they had not been observed to " gape " for 2 days.

Certain infected chicks were dosed with either 1 cc. or 2 ccs. twice daily of a 10 per cent. solution of this substance but less certain results were obtained. The reason for this was not apparent until a phrase in Chopra and Chandler's " Anthelmintics and their uses " p. 112, was noticed. They say " . . . When given in large doses most of the drug appears to pass out in the faeces, partly in combination with fats and fatty acids." It would seem therefore that 1 or 2 ccs. of a 10 per cent. solution was a too heavy dose for chicks of that age. None of them died, however, as a result of the toxic effects of the susbtance in this case.

The action of CCl_4 is very rapid. It is absorbed and excreted by the lungs within a few minutes after administration. For this reason it may be particularly useful when the patient is in the last stages of the disease as giving quick relief. But its marked toxic reaction must not be forgotten.

Reference.

Atkins, W. R. S., 1931. " The Treatment of ' Gapes ' in Chickens." (Correspondence.) *Nature Lond.*, cxxviii (3231), p. 585. (*W.L.* 14900).

Journal of Helminthology, Vol. XIII, *No.* 1, *Feb.*, 1935, *pp.* 9–12.

On Nodules occasioned by Gapeworm in Pheasants.

By Phyllis A. Clapham, Ph.D.

(*Lawrence Research Student of the Royal Society.*)
(*From the Institute of Agricultural Parasitology, St. Albans.*)

For many years past, Syngamus has been recognised as a serious trouble in both avian and mammalian hosts, and the course of the disease has been closely followed in several birds of economic importance. The symptoms are well known and have often been cited. Clinically the first symptom to appear is a characteristic sneezing and coughing in the affected bird. This is followed later by the best known symptom—the actual gaping—when the bird stretches its neck and opens its beak widely in its endeavour to ease respiration. There are minor signs such as a general debility with ruffled feathers but these are secondary and not specific to this disease. Internally in the trachea there is an increase in the secretion of mucus, which coupled with the presence of the gapeworms is sufficient to cause asphyxiation and the bird dies.

Among domestic birds, not all hosts are so severely affected. The turkey for instance, which is supposed to be the natural host of the parasite, rarely shows any signs of the disease: the host-parasite relationship seems to be perfectly adjusted. The most ill-balanced relationship seems to be in the chicken where young birds are highly susceptible and those affected frequently die at an early stage of infection. If they survive the first few weeks of life an immunity develops, the parasite is thrown off and the host continues its growth and development normally. Intermediate stages between these two conditions are present. For instance, the partridge chick frequently dies from the infestation but a balance seems to develop and a fair number of older birds are found harbouring gapeworms. In the pheasant still more adult birds have been found affected, though many young chicks die in the first few weeks of life. An interesting lesion has been noticed in this host.

A covey of pheasants, aged one month, was presented to the Institute of Agricultural Parasitology in June 1933. They had been reared under hens and all were infected with gapeworms. Of 15 birds, 6 died within the

next 6 days and post-mortem examination showed the presence of from 3–9 pairs of gapeworms in the tracheae. In March, *i.e.*, at the age of 10 months, 2 birds died from accidental causes. Gapes were present in the tracheae and in each well-developed nodules were observed at the point of attachment of the gapeworms. In June and July 2 more birds were noticed to be " gaping " very severely. Viable eggs were obtained from the faeces and when the birds died in July, at the age of 14 months, 2 and 3 pairs of worms respectively were recovered from the tracheae. Death was due to an asphyxiation caused, however, not by the gapeworms, but by the development of nodules which caused a serious blockage. In one case, there were three nodules, corresponding to three worms, each of which had reached the size of a small pea. They were obvious externally as well as internally in the tracheae. In each case the male worm was attached and the female was free. The attached males were left *in situ*, together with the anterior ends of the females which were cut below the point of union and the nodules were sectioned for histological examination.

Macroscopically the parasites looked healthy and the eggs obtained from the females produced viable embryos. There was no marked secretion of mucus. The nodules were firm in texture and of a warm red colour, indicating some inflammatory reaction. The tissues immediately surrounding the nodule were normal in appearance.

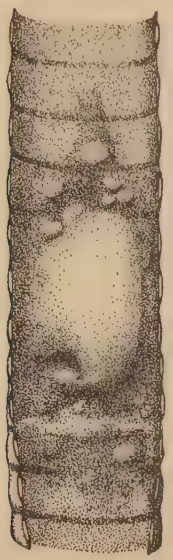
Sections showed clearly the histological reactions to the parasite and its mode of attachment.

At this stage it may be remarked that the gapeworm is able to attach itself very firmly to the mucosa of the tracheae. In chickens, small red abraded areas with a tiny papilla, due to the attachment of this parasite, have often been noticed. This petechial haemorrhage may be considered as the first lesion but as the chick rarely harbours the worm for many weeks there is little opportunity for further development. In these pheasants, however, the parasites had been present for over a year and considerable development had occurred.

The gapeworm attaches itself by drawing into its buccal cavity some of the lining membrane of the host trachea. It is often stated that the male and female attach themselves alternately ; certainly it is unusual to find both attached at one and the same time. But from the size and development of the nodules described below, it would seem either that they always attach themselves at exactly the same point or that, as the worms age, they tend to remain attached for a long time without releasing their

hold, thereby setting up a chronic irritation. The glandular cells of the lining epithelium become extremely active and produce a watery aerated secretion, particularly in young infections. Later this secretion becomes thicker and may cause the death of the parasite.

Meanwhile changes occur in the host tissues. The tissue actually held by the gapeworm in its buccal cavity is the first to show change. The epithelium disappears but whether this is due to mere mechanical abrasion



W. McDonald

Nodule formation caused by *Syngamus trachea* in the trachea of Pheasants.

or to some secretory activity is not obvious. The corium of the submucosa hypertrophies very considerably though metastatic changes do not occur. It still retains its fatty and lymphoid character. The white fibres increase and the whole region grows to such an extent as to completely surround the head and anterior end of the worm. Below this areolar layer are elastic fibres. This layer becomes active and tends to

cut off the nodule from the healthy tissue. Frequently a thick layer of yellow fibres may be present. Fibroblasts appear circumferentially and in older infestations the nodule is completely cut off by a thick fibrous layer and necrosis, followed by caseation, sets in centrally.

In two nodules the papillae had developed in the neighbourhood of one of the tracheal cartilages. Degenerative changes were apparent and considerable dissolution of the cartilage had occurred.

Throughout the whole of the nodule there was a marked inflammatory reaction with an infiltration of leucocytes. Occasional giant cells were seen in the sections.

Up to the present time nothing seems to have been known of the pathogicity of *S. trachea* and there has been a tendency to assume that tissue changes have been absent. The mucus in the trachea is frequently blood stained, due no doubt to the blood-sucking activities of the parasite. The seriousness of this habit has not yet been estimated. Ortlepp (1923) says that the first symptoms of the disease do not appear until the 9th day after infestation. Though he examined many lungs containing gapeworm larvae, nothing abnormal was apparent. Macroscopically there were no haemorrhagic spots on the surface as is the case in lungs containing Ascarid larvae. In section he saw no evidence of congestion or inflammation.

Nineteen species of Syngamus have been recorded from a variety of hosts, both avian and mammalian, including man, but the literature in all cases confines itself to morphological descriptions and to theories or experiments dealing with the life history. This record therefore of marked tissue changes seems interesting.

Reference.

Ortlepp, R. J., 1923. "The Life-History of *Syngamus trachealis* (Montagu) v. Siebold, the Gapeworm of Chickens." *J. Helminth.*, 1 (3), 119–140. (*W.L.* 11224b,)

Journal of Helminthology, Vol. XIII, *No.* 1, *Feb.*, 1935, *pp.* 13–18.

On the Occurrence of *Rhabditis coarctata* (Nematoda) on Caterpillars in the Marquesas Islands.

By J. N. Oldham, B.Sc., Ph.D.

(*Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

Early in June, 1933, the writer received two vials of caterpillars, to which nematodes were attached, from Mr. A. M. Adamson of the Pacific Entomological Survey with a request, supported by Mr. E. P. Mumford, the Director of the Survey, that an examination and identification of the helminths would be much appreciated.

The nematodes were attached to the exoskeleton of the caterpillars, which were reported as common and burrowing in the fruits of the Tahitian chestnut (*Inocarpus edulis* Forster), at various points on the body, sometimes singly sometimes in groups. The infested caterpillars were collected on 10th September, 1930 at Ihiota, Hanavave Valley, Fatuhiva in the Marquesas Islands at an altitude of 450 feet.

Some of the nematodes, which were in an encysted stage, were detached from the caterpillars, examined and measured and found to be specimens of *Rhabditis coarctata* Leuckart, 1891. From amongst the encysted forms and in the sediment in the two tubes 53 adult nematodes were obtained, 25 of which were males. From an examination of the male bursa and spicules and measurements of both male and female specimens the original identification made from the encysted forms was corroborated. All the adult specimens belonged to this species and no other parasitic or free-living forms were encountered.

This record of infestation by *R. coarctata* of caterpillars found within plant fruits is somewhat remarkable since the nematode has hitherto been recorded as occurring naturally only in sheep and cow dung and using various coprophagous insects, on which they encyst, solely for mechanical transport to fresh dung which provides conditions suitable for their further development. Accordingly the writer communicated with Messrs.

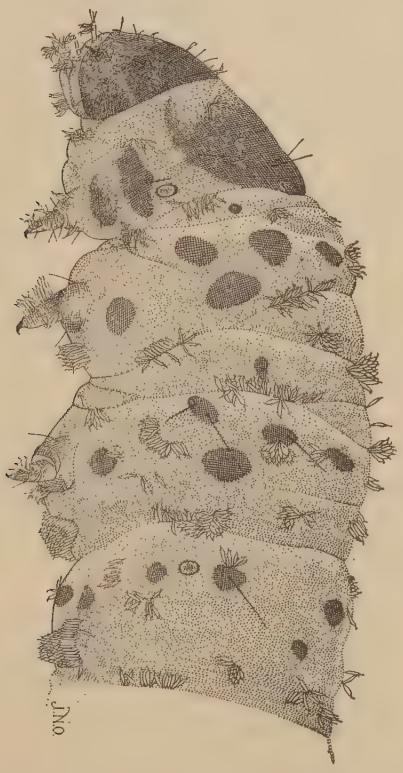
Mumford and Adamson in order to obtain what information there was available on the habits of the caterpillars. Communications with Mr. Carl Heinrich of the Division of Insects, United States National Museum, into whose hands the collection of Lepidopterous larvae had been placed, and with Mr. Edward Meyrick, England, were also made with a view to obtaining information on the identity of the infested insect. To these four gentlemen the writer desires to tender his thanks for the information they so readily gave.

Historical.

Rhabditis coarctata was first described by Leuckart in 1891 encysting on the common dung beetle, *Aphodius fimetarius* L., particularly on the tarsi and mouth-parts where they occurred in hundreds grouped together like the hairs of a brush. He described both free-living and encysted stages. In the same year Moniez published some comments on Leuckart's observations in which he disagreed on some points with the latter author's findings. In 1892 Leuckart again mentioned this species from the same host.

Little more attention seems to have been paid to this interesting nematode until 1919 when Steiner published some observations on the encystment of Nematodes in general and of *R. coarctata* in particular. His conclusions agree with later researches which show that encystment occurs so that the worms may survive through unfavourable circumstances and so continue the life-cycle when more congenial conditions arise. Later Steiner (1924) mentioned this species on *Geotrupes* sp. and another dung beetle from Falls Church, Va., this being the first record of the occurrence of the nematode in the United States of America. Chapin (1925) in a very brief note records the presence of cysts of a *Rhabditis*, similar to *R. coarctata*, attached to mites of the genus *Macrocheles*, collected from a mass of decaying fleshy fungi. Christie (1925) also found the dung beetle, *Aphodius fimetarius* L., infested with cysts of this nematode in the vicinity of Falls Church, Va., U.S.A., and was able to produce cultures of the worm in cow dung from which he subsequently produced encystment on *A. granarius* L., *A. inquinatus* Fbr., *Sphaeridium scarabaeoides* L. and *Cercyon* sp., all dung-inhabiting insects. He also demonstrated that the worms had no host selective tendencies for cysts were freely formed on *Hoplocephala bicornis*, a beetle normally found under bark of decaying stumps and logs and rarely coming into contact with dung.

The present writer, jointly with Dr. M. J. Triffitt, studied the morphology of both free-living and encysted stages of this nematode and investigated its life-history and bionomics in 1927. The material for study was encountered at Warwick, England, on several naturally infested coprophagous beetles from sheep dung. Christie's findings were confirmed as encystment occurred in cultures on beetles other than dung-frequenting species.



Head end of an infested caterpillar showing attached cysts of *Rhabditis coarctata* Leuckart, 1891. From nature, magnified.

Life-History.

The life-history of *Rhabditis coarctata* is briefly as follows. The adults occur naturally in sheep and cow dung of pasture lands and the females, following fertilisation, pass eggs in a segmented condition. These give rise to larvae which live freely in the dung, feeding and growing to the pre-encystment stage when their development is arrested. Beyond this condition they are apparently incapable of pursuing their development without the presence of some insect on which they can encyst. When, under normal conditions, beetles are present in the dung these larvae attach themselves in groups to the exoskeleton of the insects and form elongated cysts in which the larvae remain quiescent until favourable conditions for excystation are attained. On an infested insect passing to a fresh patch of dung the conditions necessary for the further development of the worms are attained. The larva then escapes from the cyst and resumes an active life. Feeding is accompanied by growth, and, after a period of time which is probably governed at least in part by the factor of temperature, the adult stage is reached.

Observations on the Specimens from the Marquesas Islands.

All the specimens submitted, amounting to 15 caterpillars, were infested with cysts in varying degrees of intensity. They were observed attached to several parts of the exoskeleton either solely or in groups. Grouping of the cysts occurred, however, more commonly in the following situations; head, especially on the maxillary palps; the thoracic and abdominal prolegs including the anal prolegs and in the intersegmental folds, particularly of the sides.

Since the nematode, as previously stated, is known in nature as a dung-frequenting species encysting on coprophagous insects the record of this association with a caterpillar appeared extremely interesting. Enquiries, however, into the habits of the caterpillars elicited some information which, unfortunately, did not go far towards explaining how the infestation might have arisen. The writer had in mind the possibility that if the caterpillars spent part of their life on the ground before proceeding to burrow into the Tahitian chestnut fruits they might thus have acquired the infestation provided the ground had been contaminated by dung either

deliberately as a result of manuring practice or accidentally as might happen if animals had been feeding or tethered in the vicinity. Mr. Adamson, *in litt.*, mentions that little was learned about the habits of the caterpillars beyond the fact that they mine the seeds of the Tahitian chestnut and pupate within them. Some of the fruits from which specimens were collected were picked up from the ground below trees growing not far from houses and it was quite possible that horses might have been tethered near these trees. He thought it unlikely, however, that the fruits would fall near dung of other animals, at least in the neighbourhood of Atuona, Hivaoa, where there are no pigs at liberty and few, if any, cattle and sheep. He also mentioned that the specimens from Fatuhiva might just as easily have come into contact with horse manure as those from Hivaoa and possibly with dung from other animals. At the same time he stated that there was no reason to believe that the caterpillars ever left the fruits and that, while the fruits might have fallen near manure, the chances were that they did not. From this information the method of infestation is thus left a matter of conjecture.

Similarly, information regarding the species of insect concerned produced little help as it was thought possible that a specific determination might be of value in tracing information in published records. Mr. Carl Heinrich mentioned that he had been unable, up to the time of writing, to determine either the species or genus concerned while Mr. Meyrick considered that the *Inocarpus* feeders belonged to the family Eucosmidae and put forward the opinion that they were either a *Laspeyresia* or a peculiar genus endemic in the Islands.

At the same time the record of this association between *R. coarctata* and a caterpillar is an interesting one and appears to be the first in which a Lepidopterous larva has been mentioned as acting, in nature, as a host for this nematode.

References.

Chapin, E. A., 1925.—In "Proceedings of Helminthological Society of Washington." 80th Meeting. *J. Parasit.*, xi (4), p. 225. (*W.L.* 11428.)

Christie, J. R., 1925.—In "Proceedings of Helminthological Society of Washington." 83rd Meeting. *J. Parasit.*, xii (2), p. 106. (*W.L.* 11428.)

LEUCKART, K. G. F. R., 1891.—" Über einen an *Aphodius fimetarius* sich verpuppenden freilebenden Rundwurm, *Rhabditis coarctata* n. sp." *Verh. deuts. zool. Ges.*, I, pp. 54–56. (*W.L.* 22270.)

——, 1892.—" Verpuppung eines Wurms." *Naturw. Wschr.*, VII (17), p. 171. (*W.L.* 14929.)

MONIEZ, R., 1891.—" Les Nymphes de Rhabditis." *Rev. Biol. du nord de la France*, III (12), pp. 470–473.

STEINER, G., 1919.—" Bemerkungen über die sogenannte Verpuppung der *Rhabditis coarctata* Leuckart und das Bilden von Zysten bei Nematoden überhaupt." *Biol. Zbl.*, XXXIX, pp. 59–65. (*W.L.* 2981.)

——, 1924.—In " Proceedings of Helminthological Society of Washington." 69th Meeting. *J. Parasit.*, X (4), p. 212. (*W.L.* 11428.)

TRIFFITT, M. J. & OLDHAM, J. N., 1927.—" Observations on the Morphology and Bionomics of *Rhabditis coarctata* Leuck. occurring on Dung Beetles." *J. Helminth*, V (1), pp. 33–46. (*W.L.* 11224b.)

Journal of Helminthology, Vol. XIII, *No.* 1, *Feb.*, 1935, *pp.* 19–24.

On *Cylindrogaster curzii* n. sp., a saprophagous Nematode.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

Early in 1934 the writer received from Prof. Mario Curzi of Rome an agar tube culture on which were growing, in addition to bacteria and a fungus belonging to the genus *Fusarium*, a number of nematodes which Prof. Curzi wished to have identified. The majority of the nematodes proved to belong to the genus *Cylindrogaster* whilst the others were found to be *Aphelenchus avenae* Bast.

The agar culture had been inoculated originally with rotting material from the sub-cortical tissues of grafts of *Manihot utilissima* Pohl (bitter cassava), growing in Italian Somaliland. According to information supplied by Prof. Curzi the rotting condition of the grafts was due, in all probability, to the excessive moisture of the surrounding soil whence the tissues had been invaded by the bacteria, fungus and nematodes. The latter were present in the tissues from the epidermis to the cambium. In view of the fact that the worms were flourishing on the agar medium there can be no doubt that they are saprophagous forms. *Aphelenchus avenae* is already known from the work of Christie (1933) to be amenable to cultivation on various agar media under conditions which point to a saprophagous mode of nutrition and its occurrence under similar conditions in this, and other cultures made by the writer, supports Christie's observations. The other two species of *Cylindrogaster* already described by the writer namely, *C. longistoma* (Stefanski) and *C. ulmi* Goodey, are also undoubtedly saprophages. The species occurring in the culture received from Rome differs from the two just mentioned in the shape of the male tail and constitutes a new species which is named after Prof. Curzi.

Morphology.

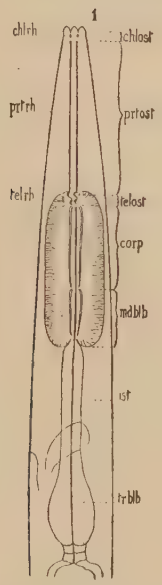
Dimensions. Female: length, 0·878 mm. to 1·43 mm., α=19·4–30, β=6·4–7·8, γ=6·4–8·8, V=48·3% to 52·7%; *Male:* length, 0·72 mm. to 1·08 mm., α=23·3–28·7, β=5·1–6·58, γ=25–32, spicules, 23μ to 26μ, gubernaculum 12μ to 14μ.

The body in both sexes tapers gradually in the eosophageal region to the head end. The female tail is long and sharply pointed whilst that of the male is short and blunt, thus presenting a marked difference from that of *C. longistoma*. The cuticle is finely striated transversely. The mouth is surrounded by 6 conical lips each of which appears to bear at least two minute papillae. The latter are, however, so small that the writer has found it impossible to determine their exact number and arrangement even under the highest magnification obtainable with an oil-immersion and a high-power ocular. It is possible that they have the same arrangement as that determined by Steiner (1933) for the circum-oral papillae of *C. longistoma*.

The buccal and oesophageal regions are arranged as in the two previously described species and their several sections can be homologized with the corresponding parts in *Rhabditis* as has been done by Steiner (l.c.) on the basis of a terminology introduced by Chitwood and Christie. The shallow buccal cavity is called the *cheilostom* and the short lateral thickenings of the walls the *cheilorhabdions*. The next section is the long tubular pharynx or *protostom*, the straight walls of which are the *protorhabdions* and in this species, as in the other two, their anterior ends are very slightly knobbed. The protostom is continued into the substance of the oesophagus for a short distance where it is ventrally flexed. There is a break at the ends of the protostom which is followed by the *telestom* in the walls of which are two short thickenings, the *telorhabdions*. Steiner states that in *C. longistoma* the telorhabdions have disappeared but in the new species they seem to be present. The muscular part of the oesophagus has the same cylindrical shape as in the two earlier species. There is a distinct break in the cuticular lining of the lumen which, according to Steiner, marks the junction of the corpus of the oesophagus with the true median bulb ; the two parts having become fused into a single structure. This junction is also very distinct in the worms which the writer described under the name of *Cylindrogaster ulmi* which, however, Chitwood (1933) has recently transferred to a new genus *Goodeyus* as *G. ulmi* because the male has a bursa and the female a single prodelphic gonad. It may be pointed out in passing that the worms described by Fuchs (1933) under the name of *Rhabditolaimus schuurmansi* are undoubtedly synonymous with *G. ulmi*.

The non-muscular region of the oesophagus is made up of a narrow neck or isthmus—about equal in length to the protostom—and a terminal pyriform swelling which contains the three oesophageal glands but lacks a

valve apparatus. The nerve ring crosses the isthmus and the excretory pore, which, as in the other two species, is very difficult to locate, opens a little in front of the terminal swelling. The intestine calls for no special description.

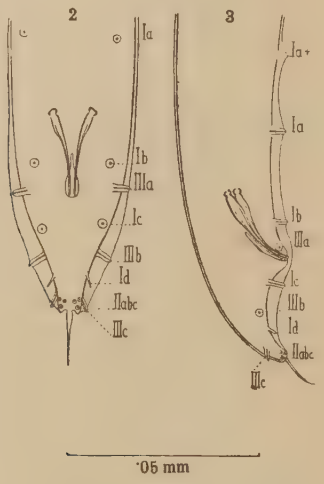


Cylindrogaster curzii n. sp.

Fig. 1.—Buccal and oesophageal region in lateral view, highly magnified to show the arrangement of the parts: *chlost*, cheilostom, *chlrh*, cheilorhabdions, *corp*, corpus of oesophageal bulb, *ist*, isthmus of oesophagus, *mdblb*, median oesophageal bulb, *prtost*, protostom, *prtrh*, protorhabdions, *telost*, telostom, *telrh*, telorhabdions, *trblb*, terminal oesophageal bulb. Magnified to same scale as figs. 2 and 3.

Female.—As the dimensions show, the vulva is practically equatorial in position; V varying from 48·3% to 52·7%. The gonads are paired, opposed and reflexed as in *C. longistoma*. The female tail is long and tapering but is perhaps not quite so filiform as that of *C. longistoma*. A small lateral papilla is found on either side of the tail a short distance behind the anus.

Male.—The gonad is single, outstretched and its anterior end is reflexed for a short distance. Behind the cloacal aperture the tail is curved a little ventrally and tapers rapidly to the terminus which is furnished with a comparatively short, narrow tail process. The cuticle of the tail is considerably inflated but does not enclose the terminal process. There are 10 pairs of caudal papillae, 9 pairs of which are innervated and are fairly



Figs. 2 & 3. Male tail in ventral and lateral view respectively, highly magnified, showing spicules, gubernaculum and distribution of the caudal papillae.

easily distinguishable. They have a distribution very similar to those of the male of *C. longistoma* as follows. A row of 4 pairs of papillae, Ia, b, c and d. Ia and Ib are pre-anal in position ; Ic and Id are post-anal. Id is shorter and narrower than the others of this series, appears to lack a central core and does not reach to surface of the cuticle. It is fairly easily distinguishable in specimens freshly killed by heat but is difficult to find in

specimens mounted in weak glycerine. Next a compact group of 3 pairs of small papillae, IIa, b and c, at the end of the tail. As a rule these are arranged as shown in fig. 2 at the angles of a triangle, the apex of which points backwards. Finally there are 3 pairs of lateral papillae, IIIa, b and c. IIIa is just pre-anal and is directed ventro-laterally, IIIb is post-anal and is also situated on the ventral side of the tail behind Ic but is laterally directed. IIIc is situated behind IIa, b and c and is directed postero-dorsally. Very rarely there is found a single extra papilla in advance of 1a, indicated as 1a+ in fig. 3.

The spicules are paired and are rather slender. From the narrow point the shaft of each gradually widens to a slight ventral prominence situated at about three-quarters of the total length from the tip. The head of each is rounded and knobbed by constriction. The gubernaculum is simple and a little more than half the length of the spicules. In ventral view it can be seen that it broadens out under the tips of the spicules and is rather spatulate in shape.

Systematics.

Filipjev (1934) has recently made *Cylindrogaster* a synonym of *Aulolaimus* de Man. The writer (1927) drew attention to the points of similarity between the pharynx and oesophagus of the two genera and at the same time indicated the differences between the caudal characters of the males. Chitwood (1933) has also discussed the matter and concludes that the male of *Aulolaimus*, in possessing median pre-anal papillae shows relationships with *Cylindrolaimus* rather than with *Cylindrogaster*. The writer sees no good reason for following Filipjev's taxonomy.

References.

Chitwood, B. G., 1933.—" On some nematodes of the superfamily Rhabditoidea and their status as parasites of reptiles and amphibians." *J. Wash. Acad. Sci.*, xxiii (11), 508–520. (*W.L.* 11600.)

Christie, J. R., 1933.—" Notes on some saprophytes or semi-parasitic nematodes infecting plants." Proc. Helminth. Soc. Wash., *J. Parasit.*, xx (1), 63–64. (*W.L.* 11428.)

Filipjev, I. N., 1934.—" The Classification of the Free-living Nematodes and their Relation to the Parasitic Nematodes." *Smithson. misc. Coll.*, lxxxix (6), 1–63. (*W.L.* 20256.)

Fuchs, A. G., 1933.—" Einige Nematoden bei *Scolytus scolytus* F." *Capita zool.*, iv (1), 1–44. (*W.L.* 5943.)

GOODEY, T., 1927.—" *Cylindrogaster coprophaga* gen et sp. nov. A Nematode found in a Culture of Faeces from a Wild Brown Rat." *J. Helminth.*, v (1), 25–32. (*W.L.* 11224b.)

——, 1930.—" A new species of the nematode genus *Cylindrogaster*." *Ibid.*, VIII (2), 89–92.

STEFANSKI, W., 1928.—" Sur l'identité des espèces *Rhabditis longistoma* Stefanski, 1922 et *Clyindrogaster coprophaga* Goodey, 1927." *Ibid.*, VI (2), 77–78.

STEINER, G., 1933.—" The nematode *Cylindrogaster longistoma* (Stefanski) Goodey, and its relationship." Proc. Helminth. Soc. Wash., *J. Parasit.*, XX (1), 66–68.

Journal of Helminthology, Vol. XIII, *No.* 1, *Feb.*, 1935, *pp.* 25–40.

The Complement Fixation Reaction in *Schistosoma japonicum* with Cercarial Antigen prepared from *Oncomelania hupensis.*

By Mary N. Andrews, M.D., D.T.M. & H.

(*Division of Pathological Sciences, Henry Lester Institute of Medical Research, Shanghai.*)

Introduction.

Since 1909 reports have been published by various workers on the serological diagnosis of Schistosomiasis by means of the complement fixation reaction. The test antigens employed were obtained either from adult worms or from Schistosome cercariae in the livers of infected snails.

Japanese workers used either alcoholic or aqueous extracts of adult *Schistosoma japonicum* worms as test antigens. In most cases paired worms (*i.e.*, both male and female) were employed, but in some instances the extracts were prepared from the male worms only.

Fujinami and Namakura (1909) obtained negative results on two calves infected with *Schistosoma japonicum*, using as antigen an alcoholic extract of adult worms.

Hayami and Tanaka (1910) reported positive results in only one infected calf using as antigen an aqueous extract of adult worms.

Yoshimoto (1910) obtained positive results with the sera of 11 persons infected with *S. japonicum*, while the sera from 19 non-schistosome cases with negative Wassermann reactions gave negative results. Three out of 10 non-schistosome sera with positive Wassermann reaction gave a positive result. The antigen employed was an alcoholic worm extract.

Tanaka (1912) tested the sera of various animals with aqueous extracts of male worms, and alcoholic extracts of worms of both sexes. Positive results were obtained with horse and rat serum using the aqueous extract, and with rat serum using the alcoholic extract. Negative findings were recorded with the sera of monkeys, dogs, mice and cattle.

Sueyasu (1916) used aqueous and alcoholic worm extracts on the sera of two experimentally infected horses. One animal which had been infected two years previously, and had apparently undergone spontaneous cure gave a positive result. The second animal had been hyper-infected two months previously and gave a negative result.

Miyaji and Imai (1928) used as antigens saline and alcoholic extracts of finely triturated dried worms of both sexes. Positive reactions were obtained in rabbits and in 6 dogs. One dog, however, gave negative reactions.

Sera were also examined from 49 persons in an infected area. Sixteen out of 17 ova-positive cases gave positive results. Out of 32 cases in which no ova were found 18 gave positive results. Three out of 25 non-schistome syphilitics gave positive results with the alcoholic extract, but all 25 gave negative results with the aqueous extract.

Fairley (1919) in Egypt found that adult worm antigens were not satisfactory for the diagnosis of infections with *Schistosoma mansoni* and *Schistosoma haematobium*, and he prepared cercarial antigens which proved more potent.

These antigens were prepared by extracting with absolute alcohol the fresh livers of *Planorbis boissyi* infected with cercariae of *Schistosoma mansoni*, or *Bullinus contortus* infected with cercariae of *Schistosoma haematobium*. These proved equally efficacious in the diagnosis of infections with either species of Schistosome.

Murray (1920) confirmed these findings using an antigen prepared from *Physopsis africana*.

Cawston (1921) stated that Murray had found the antigen equally effective for the diagnosis of *Schistosoma japonicum*, *Schistosoma haematobium*, or *Schistosoma bovis* infections.

Tanabe (1923) using an antigen prepared from *Lymnaea palustris* infected with cercariae of *Schistosoma pathlocopticum* obtained positive results in a rat infested with this species, and in two others infected with *Schistosoma japonicum*.

Fairley (1923) used an antigen made from the infected livers of *Planorbis exustus* (*Schistosoma spindalis*) on a series of goats infected with this parasite. He showed a group reaction for *Schistosoma spindalis* and *Schistosoma indicum* in cattle, and *Schistosoma haematobium* in man.

Le Bas (1922) confirmed the efficacy of cercarial antigens, but differed from Fairley as to the lipoidal character of the antigen. She regarded it as protein in nature.

Fairley (1925) adhered to his view that it was a lipoid or a lipoidal complex.

Fairley (1926) and Fairley and Jasudasin (1930) reported favourable results using the test on goats and human cases.

Fairley (1933) published results showing positive reactions in goats infected with *Schistosoma mattheei* and *Schistosoma bovis* using as antigen a *Schistosoma spindalis* cercarial extract.

The conclusion he reached was that there was an antigenic complex common to the mammalian Schistosome cercariae: the *Schistosoma spindalis* cercarial antigen being efficacious in the diagnosis of infections with the three human Schistosomes and four species occurring in cattle.

In Fairley's experience other helminthic infections have not given positive reactions with Schistosome cercarial antigens, but Cawston (1921) recorded positive reactions in sheep infested with *Fasciola hepatica* when schistosome antigens were used.

Hoeppli (1921) obtained a positive reaction in one case infected with *Schistosoma mansoni* using as antigen an alcoholic extract of *Fasciola hepatica*. However, Bettencourt and Borges (1922) and Le Bas (1924) were unable to confirm this, and did not find such extracts of any value in the diagnosis of human Schistosomiasis, although they agreed that human sera which were Wassermann positive might give positive reactions.

Comparatively little use has been made of the complement fixation reaction in the diagnosis of infections with *Schistosoma japonicum*. In Japan, as already noted, adult worm antigens have been used in a few cases, and it has been shown that other schistosome cercarial extracts will give a positive result in cases of infection with *Schistosoma japonicum*.

There are no records of the test having been employed in China where the disease is common and widespread. Dr. Fairley suggested to the writer the possibility of manufacturing an antigen from the infected livers of the molluscan host, *Oncomelania hupensis*, and he also presented some bilharzial antigen which was used in the earlier cases of this series while the specific antigen was being prepared, and also as a control for the antigen made from Oncomelania.

Other trematode infections such as *Clonorchis sinensis* and *Fasciolopsis buski* are endemic in certain parts of China, and although Shanghai is not in the endemic region for these parasites, yet cases are from time to time admitted to the hospitals, and we have had the opportunity of examining the sera, so far with negative results.

In addition to cercarial antigens from *Oncomelania hupensis* we have now prepared antigens from adult Schistosome worms, and from *Fasciola hepatica*, and these are being used in the test. It is hoped to publish these results later when a further series of cases has been examined.

Author's Investigations.

Oncomelania hupensis snails have been collected from various areas around Shanghai. A total of 52,834 snails have been examined for infection with Schistosome cercariae. The average infective rate was 13·3 per 1,000.

At first the snails were crushed and the liver teased in saline and examined for the presence of cercariae. This method proved wasteful as only livers containing a large number of mature cercariae are suitable for use in the manufacture of antigen, and livers containing sporocysts or developing cercariae had to be discarded. As it takes nine weeks for the cercaria to develop in the molluscan host it is obvious that there must be a wastage when the snails are killed and only examined on one occasion.

This method of examination is therefore no longer used, but the snails are kept alive in the laboratory under conditions which as nearly as possible approximate to their natural living conditions.

Oncomelania is a semi-aquatic mollusc usually found in the damp soil on the edges of creeks above the water level. When placed in water the mollusc will gradually climb up above the water level.

In the laboratory they are put in flat dishes containing moist earth, obtained if possible from an endemic region and some blades of grass are planted in the dish. At weekly intervals the snails are transferred to test tubes containing 5 ccs. of water and left to stand overnight. In the morning they are examined with a hand lens for the presence of cercariae, and infected individuals are put aside to be used in the manufacture of antigen.

After examination the snails are fed and then transferred to the earth in the dish again.

Originally the food given was boiled finely minced lettuce, but this did not prove a suitable food, and many of the snails died. They were therefore allowed to feed on the faecal deposit which collects at the bottom of the flask when faeces are examined for Schistosome ova by Meleny and Faust's technique. We have experienced no difficulty in keeping the snails alive for months in the laboratory under these conditions.

Attempts have been made to infect snails artificially in the laboratory. Only snails which after nine weeks' observations have shown no cercariae have been used for this purpose. Infection has been attempted either by placing the snails in water containing miracidia, the snails being placed

singly in water containing 5 or 6 miracidia, or they have been allowed to feed on faeces containing viable schistosome ova. Although miracidia have been seen to penetrate the soft parts of the snails, they do not in all cases develop to cercariae, as we have only found cercariae in 4 per 1,000 of the snails so treated.

Locality.	Number of Snails examined.	Number infected.	Rate per 1,000.
Soochow—			
Area No. 1	23,980	402	16·7
,, 2	19,313	78	4·0
Kashing	3,157	164	51·9
KanT'eo	1,484	5	3·3
Henli	4,910	54	11·0
	52,834	703	

Average infective rate per 1,000—13·3.

Manufacture of Antigen from Oncomelania hupensis.

Fairley's technique for the preparation of cercarial antigen was used, with certain modifications which were necessary owing to the fact that we were dealing with a very small mollusc.

A comparison of *Oncomelania hupensis* with Planorbis shows that the latter is at least twenty times as large as Oncomelania. The liver of Oncomelania is extremely small only weighing 0·005 gram. It was estimated that twenty livers of this snail were approximately equivalent to one Planorbis liver, so that twenty infected livers should be extracted in 1 cc. of Absolute Alcohol in order to obtain an antigen of the same strength as that prepared from Planorbis. It was, however, not found practicable to prepare so concentrated an extract, as owing to loss of fluid on the side of the tube during shaking and a further small loss during filtration, the minimum amount of fluid with which we could work was 2 ccs. This would necessitate the use of 40 infected livers each time. In spite of the fact that a large number of snails were examined daily and the positives put aside and kept for antigen, yet we never had more than 30–35 snails available for use at a time. We therefore decided to make the extract in the proportion of 10 livers to each cc. of alcohol.

At first the antigen was incubated for 24 hours at 37°, but this was afterwards altered to 72 hours as an attempt to increase the antigenic power.

The technique used in the present work was :—After examination positive snails were put aside and kept until at least twenty positive individuals had been obtained. These were then gently crushed with a glass rod and the livers dissected out and immediately transferred to Absolute Alcohol in the proportion of 10 livers to each cc. They were thoroughly teased and the extract shaken well for twenty minutes. It was then incubated at 37 degrees for 72 hours, being thoroughly shaken each day. After removal from the incubator it was again shaken for twenty minutes and then either filtered or else centrifuged and the clear fluid pipetted off. The clear amber coloured fluid was then stored in the ice chest. When about 30 ccs. of antigen had been prepared it was concentrated in a water bath at 45°C. until the fluid became turbid, when it was cleared by the addition of Absolute Alcohol, put up in ampoules and stored in the ice chest until required for use.

Mr. W. Y. Lee of the Division of Physiological Sciences tested the extract for the presence of protein. He reported that Milton's test, Ehrlich's diazo reaction, the Hopkins Cole reaction, and the Biuret test were negative, but that the Nitric acid ring test and the Xanthoproteic reaction were positive. He concluded that there was some protein in the antigen probably associated with a lipoid.

Dr. Li Yuan Po tested the antigen before it was used for routine tests and reported that the antigenic titre was 1–200, the anti-complementary dose 1–5, and there was no haemolytic tendency. In performing the tests he found it necessary to use the antigen in a dilution of 1–20, but even with this dilution no pseudo-positive reactions were recorded.

The routine tests were carried out under the supervision of Dr. Li. The technique employed was similar to that described by Fairley, the Bilharzial antigen being used in a 1/40 dilution. It was however, necessary to use the *Schistosoma japonicum* antigen in a 1/20 dilution. Three, five and seven minimum haemolytic doses of Complement were used and negative and positive Schistosome sera were included in the test.

In the table ++++ indicates no haemolysis, +++ slight haemolysis, ++ haemolysis up to 50%, and + almost complete haemolysis ; ± indicates complete haemolysis. Sera from 49 human beings and five dogs have been examined, a total of 125 tests being performed. Bilharzial antigen was used in 68 of the tests and the Oncomelania antigen in 57. The results are shown in the accompanying table.

TABLE OF COMPLEMENT FIXATION REACTIONS IN *SCHISTOSOMA JAPONICUM* INFECTIONS.

Hospital number.	Date.	Bilharzial antigen (Fairley) M.H.D.				Oncomelania antigen (Andrews) M.H.D.				Faecal findings.
		3	5	7	C	3	5	7	C	
(1) 1280										Hookworm and *S.j.* Ova on admission
	12–10–32	+++	++	+	–					No ova
	16–11–32	+++	++	++	–					,,
	28– 2–33	++++	++++	+++	–	++++	+++	+++	–	,,
	27– 3–33	+++	+	–	–					,,
(2) 1281										Hookworm, Ascaris and *S.j.* Ova on admission
	16–11–32	++++	++++	++++	–					*S.j.* ova
	28– 3–33	++++	++++	++++	–	++++	++++	++++	–	No ova
	27– 3–33	++++	++++	+	–					,,
	6– 7–34					++++	–	–	–	,,
(3) 1282										Hookworm and *S.j.* ova on admission
	16–11–32	++++	++++	++++	–					*S.j.* ova
	28– 2–33	++++	++++	++++	–	++++	++++	++++	–	No ova
	27– 3–33	++++	++++	++++	–					,,
(4) 1474	17– 5–32	++++	++++	++++	–					Hookworm and *S.j.* ova
	16–11–32	++++	++++	++++	–					*S.j.* ova
	28– 2–33	++++	+++	+	–	++++	+++	++	–	,,
	27– 3–33	–	–	–	–					No ova

TABLE OF COMPLEMENT FIXATION REACTIONS IN *SCHISTOSOMA JAPONICUM* INFECTIONS—*continued.*

Hospital number.	Date.	Bilharzial antigen (Fairley) M.H.D.				Oncomelania antigen (Andrews) M.H.D.				Faecal findings.
		3	5	7	C	3	5	7	C	
(5) 3148	14–10–32	−	−	−	−					*S.j.* ova
	24–10–32	−	−	−	−					No ova
	16–11–32	++++	+++	+++	−	+	−	−	−	,,
	28– 2–33	+	−	−	−					,,
	27– 3–33					−	−	−	−	,,
(6) 2736	12–10–32	−	−	−	−					*F. buski* and *S.j.* ova
	16–11–32	++++	+++	+++	−					*S.j.* ova
	20– 1–33	++++	+++	++	−					,,
	28– 2–33	++++	+++	++	−	+++	+	±	−	,,
	28– 3–33	−	−	−	−					,,
	7– 7–33	−	−	−	−					No ova
	21– 8–33	+	−	−	−	−	−	−	−	,,
	8– 1–34					−	−	−	−	,,
(7) 2870	14– 9–32	++++	−	−	−					Hookworm and *S.j.* ova
	16–11–32	+++	++	+	−					*S.j.* ova
	19– 1–33	++++	++++	++++	−					,,
	28– 2–33	++++	++++	++++	−	++++	++	±	−	,,
	27– 3–33	++++	++++	++++	−					,,
(8) 3710	15–11–32	++++	++++	++++	−					No ova
	28– 2–33	+	±	−	−	+	−	−	−	,,
	28– 3–33	−	−	−	−					*S.j.* ova
	14– 7–33	+	±	−	−	+	−	−	−	No ova
	12–10–33	−	−	−	−	−	−	−	−	,,
	29– 1–34					−	−	−	−	*S.j.* ova
	29– 2–34					++	−	−	−	No ova
	25– 6–34					−	−	−	−	*S.j.* ova

TABLE OF COMPLEMENT FIXATION REACTIONS IN *SCHISTOSOMA JAPONICUM* INFECTIONS—*continued*.

Hospital number.	Date.	Bilharzial antigen (Fairley) M.H.D.				Oncomelania antigen (Andrews) M.H.D.				Faecal findings.
		3	5	7	C	3	5	7	C	
(9) 737	24– 3–33	++++	++++	++++	–	++	+	–	–	*S.j.* ova
	16– 6–33	++++	++++	++++	–	++++	++++	++++	–	No ova
	7– 7–35	++++	++++	+++	–	++++	++++	+++	–	,,
	28– 7–33	++++	++++	+++	–	+++	–	–	–	,,
(10) 267	2– 2–33	–	–	–	–					Hookworm ova No *S.j.* ova
(11) 287	10– 2–33	–	–	–	–					*Ascaris* ova No *S.j.* ova
(12) 3213	14–10–32	–	–	–	–					Syphilis. No ova
(13) 3482	2–12–32	–	–	–	–					No ova
(14) 14628	3–11–32	++++	++++	++++	–					*S.j.* ova
(15) Chou	4–11–32	++++	++++	++++	–					*S.j.* ova
(16) 13	19– 1–33	++++	+++	+++	–					Hookworm and *S.j.* ova
(17) 3875	20– 1–33	++	++	+	–					*Ascaris*, Hookworm *Trichuris* and *S.J.* ova
(18) 1967	14– 7–33	–	–	–	–					No ova
(19) 790	9– 5–33	++++	++++	++++	–					*S.j.* ova
	16– 6–33	++++	+++	–	–	++	–	–	–	No ova

TABLE OF COMPLEMENT FIXATION REACTIONS IN *SCHISTOSOMA JAPONICUM* INFECTIONS—*continued*.

Hospital number.	Date.	Bilharzial antigen (Fairley) M.H.D.				Oncomelania antigen (Andrews) M.H.D.				Faecal findings.
		3	5	7	C	3	5	7	C	
(20) 500	18– 4–33	±	–	–	–					*S.j.* ova
	16– 6–33	++++	+++	–	–	++	–	–	–	No ova
(21) 1931	17– 7–33	–	–	–	–	–	–	–	–	*S.j.* ova
(22) 1933	21– 6–33	–	–	–	–	–	–	–	–	No ova
(23) 1791	29– 6–33	–	–	–	–	–	–	–	–	*S.j.* ova
(24) 1688	4– 7–33	–	–	–	–	–	–	–	–	*S.j.* and *Clonorchis* ova
(25) 9153	20–12–33	++++	++++	++++	–	++++	++++	++++	–	*Ascaris*, Hookworm, *Trichuris* and *S.j.* ova
(26) 3206	31–10–33	–	–	–	–	–	–	–	–	*Clonorchis* ova No *S.j.* ova
(27) 1971	31–10–33	–	–	–	–	–	–	–	–	No *S.j.* ova
(28) 1982	4– 8–33	++++	+	–	–	++++	+	–	–	*Ascaris*, Hookworm, *Trichuris* and *S.j.* ova
(29) 1937	19– 7–33	–	–	–	–	–	–	–	–	*S.j.* ova
(30) 3078	18–10–33	–	–	–	–	–	–	–	–	*S.j.* ova
	25–11–33	–	–	–	–	–	–	–	–	,,
	5–12–33	–	–	–	–	–	–	–	–	No ova
	3– 1–34	±	–	–	–	–	–	–	–	,,
(31) 3389	18–11–33	++++	+++	+	–	++++	+++	+	–	*S.j.* ova

TABLE OF COMPLEMENT FIXATION REACTIONS IN *SCHISTOSOMA JAPONICUM* INFECTIONS—*continued*.

Hospital number.	Date.	Bilharzial antigen (Fairley) M.H.D.				Oncomelania antigen (Andrews) M.H.D.				Faecal findings.
		3	5	7	C	3	5	7	C	
(32) 3295	18–11–33	++++	++++	++++	–	++++	++++	++++	–	*S.j.* ova
(33) Hangchow case	18–11–33	±	–	–	–	±	–	–	–	No record
(34) 3762	3– 1–34					+	–	–	–	*S.j.* ova
(35) 3722	3– 1–34					++++	++++	++++	–	*S.j.* ova
(36) 1441	25– 6–34 1– 1–34 7– 7–34					– – –	– – –	– – –	– – –	*S.j.* ova ,, ,,
(37) 1371	25– 6–34					–	–	–	–	*S.j.* ova
(38) 153	25– 6–34					++++	–	–	–	*S.j.* ova
(39) 1616	25– 6–34					–	–	–	–	*F. buski* ova
(40) Kashing 10	25– 6–34					++++	+++	–	–	Clinically *S.j.* No record of faecal findings
(41) Kashing 222	25– 6–34					++++	++++	+	–	Clinically *S.j.* No record of faecal findings
(42) Kashing 225	25– 6–34					–	–	–	–	Clinically *S.j.* No record of faecal findings
(43) Kashing 242	25– 6–34					++	–	–	–	Clinically *S.j.* No record of faecal findings

TABLE OF COMPLEMENT FIXATION REACTIONS IN *SCHISTOSOMA JAPONICUM* INFECTIONS—*concluded.*

Hospital number.	Date.	Bilharzial antigen (Fairley) M.H.D.				Oncomelania antigen (Andrews) M.H.D.				Faecal findings.
		3	5	7	C	3	5	7	C	
(44) Soochow 702	25– 6–34					+	±	–	–	*S.j.* ova
(45) 1768	6– 7–34					–	–	–	–	*F. buski*
(46) 1752	6– 7–34					–	–	–	–	*S.j.* ova
(47) Soochow 764 A10	13– 7–34					++++	–	–	–	Clinically *S.j.* No record
(48) 10499	13– 7–34					–	–	–	–	*Clonorchis*
(49) 1724	13– 7–34					–	–	–	–	*Ascaris* and *Trichuris*
Animal number. (1) Dog 1	6– 7–34					++++	–	–	–	Artificial infection *S.j.* ova
(2) Dog 893	23–12–32	++	+	–	–					*S.j.* ova
(3) Dog 858	23–12–32 30–12–32	++ ++	+ ±	– ± anti-complementary	– +					*S.j.* ova ,,
(4) Dog 894	22–12–32	–	–	–	–					No ova
(5) Setter	20–12–32	–	–	–	–					No ova

Discussion.

An analysis of the above results shows that of the 49 human cases 31 were ova positive for *Schistosoma japonicum*. In 6 cases there is no record of the faecal findings ; these cases were sent to us from hospitals outside Shanghai with a diagnosis of Schistosomiasis, and they are therefore included in the Schistosome positive cases, giving a total of 37 cases. Of these 37 cases 24 gave a positive complement fixation reaction and 2 others a very weak reaction.

The 12 non-schistosome cases included 2 infections with *Clonorchis sinensis*, and 2 with *Fasciolopsis buski*, besides infections with nematodes such as Ascaris and hookworm. These 12 cases all gave a negative complement fixation reaction.

There were 13 cases of Schistosomiasis with a negative reaction. In some of these the test was only done on one occasion, and reference to the table shows that in some of the ova positive cases in which a positive reaction was obtained the test was on occasions negative. Had it been possible to repeat the test on these negative cases, positive results might have been recorded on subsequent examinations. Many of these negative cases were very old standing infections with a history of from 5 to 22 years, and this fact may to some extent explain the negative findings.

We have not had the opportunity of examining any cases in the earliest stage of the disease as Chinese patients do not as a rule present themselves for treatment until the disease is well established. Most of the cases leave hospital when the symptoms are alleviated, and are lost sight of. Case 1281 has, however, been under observation for nearly two years and still has a positive reaction, although not so strongly positive now as it was 15 months ago. Case 2736 had a negative reaction for 11 months and no ova were found in the stools for 10 months. With the exception of this one case the others were not under observation for a long enough period to show whether the reaction becomes negative with cure, although on discharge some of them had no ova and a negative complement fixation reaction.

Five sera were examined from dogs. Two of these dogs had a natural infection with Schistosomiasis and gave a weak positive complement fixation reaction. One was an experimentally infected animal and also gave a positive reaction. The other two dogs were not infected with Schistosomiasis and gave negative findings with the complement fixation reaction.

In 24 cases both antigens were used and the results with the Bilharzial and Oncomelania antigen corresponded very closely, although in three instances the results obtained with the Oncomelania antigen were not so strongly positive as those obtained with the Bilharzial antigen.

In 33 cases the Bilharzial antigen was used. There were 25 cases of Schistosomiasis in this series of which 19 gave a positive complement fixation reaction.

In 40 cases Oncomelania antigen was used. There were 32 cases of Schistosomiasis in this series of which 23 gave a positive complement fixation reaction and 3 gave a weak positive reaction.

In addition to these tests 50 strongly positive Wassermann sera were tested with the Bilharzial antigen, and all gave a negative result.

These findings show that the cercarial antigens used are specific for *Schistosoma japonicum* as no positive results have been obtained either with Syphilitic sera, nor with infection with other helminths. But it would seem that a negative reaction does not necessarily exclude infection with Schistosomes, as 13 of the cases in this series gave a negative complement fixation reaction, although ova were present in the stools.

Summary.

1. The preparation of a cercarial antigen from infected livers of *Oncomelania hupensis* the intermediate host of *Schistosoma japonicum* in China is described.

2. The sera of 49 human beings and five dogs have been investigated for the Schistosome complement fixation reaction, using as antigens Bilharzial cercarial antigen, and an alcoholic extract of the livers of *Oncomelania hupensis* infected with cercariae of *S. japonicum*.

3. Thirty-seven of the 49 sera examined were from cases of Schistosomiasis, and of these 24 gave a positive complement fixation reaction, and 2 others a weak reaction.

4. Of the five dogs examined three were infected with *Schistosoma japonicum*. These gave positive reactions.

5. In 24 cases both antigens were used, and the results obtained were almost identical.

6. The Bilharzial antigen was used in 33 cases including 25 cases of Schistosomiasis, of which 19 gave a positive reaction.

7. In 40 cases the Oncomelania antigen was used. This series included 32 cases of Schistosomiasis of which 23 gave a positive reaction and 3 a weak positive reaction.

8. Negative results were obtained with 50 strongly positive Wasserman sera, and with 2 cases of infection with *Fasciolopsis buski*, and two cases of *Clonorchis sinensis*. Ascaris and hookworm infestations also gave negative results.

Acknowledgments.

I wish to express my thanks to Dr. N. H. Fairley for the gift of the Bilharzial antigen, and for advice on the work; to Dr. R. C. Robertson, Head of Division of Pathological Sciences, for his help in the collection of snails and advice during the investigation; to Dr. Li Yuan Po who undertook the serological work. Mr. S. Y. Liu and Mr. K. K. Kiang rendered valuable assistance on the technical side.

References.

Bettencourt, A. & Borges, I., 1922.—" Reaction de fixation dans la *Bilharziose vesicale* avec antigene de *Fasciola hepatica*." *C. R. Soc. Biol.*, lxxxvi, p. 1053. (*W.L.* 6630.)

Cawston, F. G., 1921.—" Bilharzia-infested snails and their employment as antigen." (Correspondence.) *Lancet*, 1, p. 250. (*W.L.* 11995.)

Fairley, N. H., 1919.—" The discovery of a specific complement fixation test for bilharziasis and its practical application to clinical medicine." *Jour. Roy. Army Med. Corps*, xxxii, p. 449. (*W.L.* 11035.)

— 1925.—" Experimental studies in bilharzia antigen. Part 1. The lipoidal nature of cercarial antigen." *J. Path. Bact.*, xxviii, p. 591; *ibid*, 1927, xxx, p. 97. (*W.L.* 11431.)

— 1926.—" The serological diagnosis of *Schistosomum spindale* (cercarial antigen)." *Arch. Schiffs-u. Tropenhyg.*, xxx, p. 372. (*W.L.* 1804.)

— 1933.—" Bilharzia complement fixation reaction on goats injected with *Schistosoma mattheei* and *Schistosoma bovis*." *J. Helminth.*, xi, p. 181. (*W.L.* 11224b.)

— & Jasudasan, 1930.—" The Complement Fixation Reaction with Cercarial Antigen." *Indian med. Res. Mem.*, 17, p. 73. (*W.L.* 9945a.)

— & Williams, F. E., 1923.—" Observations on the complement fixation reaction in liver fluke (*Fasciola hepatica* L.) infection." *J. Path. Bact.*, xxvi, p. 19. (*W.L.* 11431.)

Fujinami, A. & Nakamura, H., 1909.—*Kyoto Igakai Zasshi* (*Kyoto Med. J.*), 6, No. 4. (*W.L.* 11965a.) (Quoted from Miyaji & Imai.)

Hayami & Tanaka, M., 1910.—*Kyoto Igakai Zasshi* (*Kyoto Med. J.*), 7, No. 4. (*W.L.* 11965a.) (Quoted from Miyaji & Imai.)

Hoeppli, R., 1921.—" Die Diagnose pathogener Trematoden durch Blutuntersuchung." *Arch. Schiffs-u. Tropenhyg.*, xxv, p. 365. (*W.L.* 1804.)

Le Bas, G. Z. L., 1922.—" On the nature of the antigen in the complement fixation test for Bilharziasis." *J. trop. Med.* (Hyg.), xxv, p. 49. (*W.L.* 11587.)

— 1924.—" A note on the employment of *Fasciola hepatica* antigen for the serum diagnosis of Bilharziasis." *Proc. Roy. Soc. Med.*, xvii, p. 6. (*W.L.* 16916.)

Miyaji, S. & Imai, B., 1928.—" Serologische Studien bei *Schistosomiasis japonica*." *Zbl. Bakt.*, I. Abt. Orig. cvi, p. 237. (*W.L.* 23684.)

Murray, W. A., 1920.—" A note on a specific complement fixation test for Bilharziasis." *S. Afr. med. Rec.*, xviii, p. 168. (*W.L.* 20360.)

Suevasu, Y., 1916.—" Complement fixation in horses affected with Schistosomiasis." *Kyoto Igakai Zasshi* (*Kyoto Med. J.*), xxii, p. 188. (*W.L.* 11965a.)

Tanabe, B., 1923.—" Complement fixation test of *Schistosomatium patholocopticum* and its group reaction with *Schistosoma japonicum*." *J. Parasit.*, ix, p. 230. (*W. L.* 11428.)

Tanaka, M., 1912.—" The complement fixation test for *S. japonicum*." *Chingai Iji Shimpo*, Tokio, xxxiii, p. 721. (Quoted by Tanabe.)

Yoskimoto, M., 1910.—" Ueber die Komplementbindungs reaktion bei der Schistosomum-Krankheit in Japan." *Z. ImmunForsch.*, v, p. 438. (*W.L.* 23456.)

Journal of Helminthology, Vol. XIII, *No.* 1, *Feb.*, 1935, *pp.* 41–58.

Ecological Studies on *Nematodirus* species in Sheep in Manawatu district, New Zealand.

By J. H. Tetley, M.Agr.Sc.
(*Zoology Department, Massey Agricultural College, Palmerston North, New Zealand.*)

Introduction.

The study of populations of animals has received the attention of a large number of workers. Of these, however, but a few have been interested in the problem in its application to parasitic nematodes and in this field there is consequently a limited literature. Particularly is this so when the parasitic nematodes of sheep are considered, the incentive for study of which, in most cases, has been the pressing need for their destruction. This desire has been fulfilled to a greater or lesser degree, but at the expense of researches which would have contributed to a more fundamental knowledge of the ecological relationships of parasitic worms in general.

Few workers have attempted to portray the series of fluctuations to which the worm population within the host is subject under normal conditions of reinfection. The relevant literature so far as the writer can determine, is confined to the results of post mortem examinations contained in accounts of surveys carried out in various regions from time to time. Such information may be regarded as relevant to the static aspects of worm numbers and is valuable, but it does little to indicate the seasonal or other fluctuations in numbers which may occur in the one host over a period of time. It is for this reason that the writer has chosen to approach the question from its dynamic aspects, and has attempted to follow the rhythmical course of nematode numbers.

The matter of the inter-relationship between host and parasite has attracted little attention in respect to parasitic infection of sheep, only in the case of infection by *Haemonchus* has this been studied in any detail. It has been shown that an acquired immunity may develop, following infection by *Haemonchus contortus*, but for no other species of nematode of sheep has this been definitely established. The writer has been unable to find reference in the literature to any account of the manner of distribution of nematodes in the small intestine of the sheep. In some circumstances the total numbers of worms found in the small intestine have been

recorded but writers have been content to refer only to the general area of distribution of the species treated.

There is no exact record of the time of introduction of *Nematodirus* spp. into New Zealand. Gilruth records that *Strongylus filicollis* was present in sheep in this country in 1901.

It is certain that *Nematodirus* spp. must have been introduced in infected sheep imported either from Australia or Great Britain or from both countries sometime since their introduction in 1814, for there were no indigenous ruminants from which infection could have been contracted. The genus is now distributed throughout the country.

Identification of the Various Stages of *Nematodirus*.

Eggs.

The eggs of *Nematodirus* spp. are characteristic in several respects. Their dimensions, a minimum length of 130 μ and diameter of 65 μ are considerably greater than those of other nematodes of the alimentary canal of the sheep. In addition, they for the most part possess stout shells of 3–4 μ in thickness in which, at the time of deposition on the ground, segmentation has progressed not beyond the 6 to 8 cell stage.

The matter of the identification of the eggs of different species of *Nematodirus* was simplified in that two species *N. filicollis* and *N. spathiger* so outnumbered *N. abnormalis* and *N. helvetianus* that these latter two were ignored in the counts. Eggs of *N. filicollis* and *N. spathiger* may be distinguished readily. In the case of *N. filicollis* they possess an evenly oval shell 3 μ in thickness at the sides, and but very slightly thicker at the poles. The writer found that the dimensions of the greater numbers fell between the limits of 140 to 165 μ × 70 to 85 μ and that very few approached the limits in dimensions—130 to 210 μ × 70 to 90 μ and 130 to 210 μ × 65 to 95 μ—mentioned by Baylis and Boulenger respectively. The eggs of *N. spathiger* tend to be more pointed at the poles than those of *N. filicollis* and often are not so evenly oval. The shell is thicker, being 3 to 4 μ at the sides and about 5 to 8 μ at the poles, in this species.

The writer found that the size of the majority of eggs of *N. spathiger* fell within the limits 180 to 210 μ × 90 to 105 μ and that the range of dimensions 150 to 220 μ × 80 to 110 μ quoted by Baylis was not exceeded.

Larvae.

Immature parasitic stages of *Nematodirus* spp. may be differentiated from those of all other species occurring in the sheep by virtue of the

characters of the tail. In both larval and adult females the blunted tail tip is provided with an apical spine, absent in other genera. In males, the rudimentary spicule and bursa early show the characteristics of the adults of the genus. Young adult females were distinguishable from mature females on account of the absence of eggs. They were not confused with females which had ceased laying for in such cases obvious signs of degeneration were present.

The paler spicules of immature adult males enabled their ready separation from mature forms in which the spicules were much darker in colour.

Adults.

In the case of males, identification was readily accomplished by means of characters of the bursa, and the forms of the spicules. The slender finger like rays of the former and the length of the latter, greater than 600 μ, at once serving to distinguish the genus from others found in the sheep. Individual species of *Nematodirus* were easily separated on the form of the spicules. Females were distinguished from those of other genera on account of the large size of their eggs, and the blunted tail with its appended apical spine. Worms of this genus could with reasonable certainty be identified from other genera occurring in the small intestine of the sheep by means of the naked eye. They are larger than other species, while in the case of females, on account of the large size of the eggs they contain, they are slightly swollen in the posterior portion, in manner such as to make them resemble small editions of the whip-worm. In addition to this the anterior portion of the body in freshly collected specimens tended to curl in a spiral fashion, reminiscent of the tendrils of some climbing plants.

When making counts of the relative numbers of the individuals of the various species of *Nematodirus*, the numbers of the males only were recorded, for in the case of females the close resemblance between species made identification difficult, and inconveniently slow.

Rectal specimens of dung were obtained by means of the method adopted by Veglia, of inserting the finger, and egg counts made from these by a dilution method devised by Stoll and adopted for use on the faeces of sheep by Mönnig.

Each egg count record was expressed in terms of number of eggs found per gram weight of fresh faeces. No attempt was made to estimate the dry matter content of faeces nor was the daily output of dung recorded

for any sheep. In cases where the course of *Nematodirus* infection was being observed in an individual sheep, egg counts were made, on an average, at fortnightly intervals.

Post mortem examinations were made in sheep slaughtered on Massey Agricultural College farm and also from the surrounding district. These latter had been slaughtered at the Palmerston North Municipal abattoirs and Longburn Freezing Works.

In the majority of cases data were obtained from animals which had been freshly slaughtered, the small intestine being removed with a minimum of disturbance. In some cases lambs examined in the summer and later in the season had been drenched with various vermicides, and there was the possibility that these may have been a complication. A number of post mortem examinations were conducted on recently drenched animals, but no indication was presented that the numbers in these were less than in cases where no drenches had been given. Egg counts conducted on four lambs immediately before drenching each with 5 ccs. tetrachlorethylene, and then 6 days afterwards, showed that there had been no apparent diminution in the numbers of eggs passed, and presumably the adult worm population had not been altered.

	Nematodirus egg counts before drenching.	*Nematodirus* egg counts 6 days after drenching.
1.	50 per gram	50 per gram
2.	350 ,,	366 ,,
3.	—	—
4.	50 ,,	50 ,,

Again the egg count records of undrenched animals in which the course of *Nematodirus* infection had been observed, produced no evidence that worm numbers behaved in a different fashion from those in drenched animals examined by post mortem. The close of *Nematodirus* infection in these undrenched lambs coincided with that in drenched animals.

The contents of the small intestine were removed by running between the fingers, and examinations were conducted under low power binoculars, using petri dishes. Occasionally, when the ingesta were deeply pigmented, or opaque, fine sieves were used, and if numbers of worms were large, dilution methods were adopted.

In cases where the distribution of *Nematodirus* within the sheep was observed, the small intestine was ligatured at short intervals before attempt was made to disengage it from the mesentery. Following this,

the separation was effected, and a further series of ligatures at 3 or 5 ft. were then superimposed upon the first series. By this means the regional distribution of *Nematodirus* spp. in the intestine was preserved.

The contents of each section were conveyed to numbered jars by running the portion of intestine between the fingers. Worms for permanent collection were fixed, using the standard method, in hot glycerine alcohol, 5 parts glycerine to 95 parts of 70% alcohol.

In cases where an entire infection was being studied, it was found necessary to make temporary slides, and in these cases, fixing for rapidity was performed in cold glycerine alcohol. The worms were mounted in glycerine jelly, and up to 20 individuals per $\frac{3}{4}$ inch circular cover slip were possible, without interfering with the ease of examination. It was necessary to ring these mounts with lacquer. By using cardboard labels at each end of a slide, storage in a compact manner was facilitated without risk of damage to the mounts.

Maintenance of Sheep Free from Infection.

Lambs were reared free of infection by nematodes by separating them from the ewes within 48 hours of birth. They were maintained on lawns on which sheep had not previously grazed, and fed with cow's milk. It was found possible to keep lambs free from *Nematodirus* spp. in this manner for 10 months.

The Experimental Paddock.

The sheep in which the process of *Nematodirus* infection was observed by means of egg counts, were maintained in a paddock approximately 1 acre in extent, which formerly had been used for normal flock grazing purposes. It therefore carried an initial infection of *Nematodirus* spp. The area was situated for the most part on the sloping edge of a terrace, sandy in nature, and not of heaviest sheep carrying capacity. The topography of the paddock ensured good drainage and at no time did the area become water logged. During each summer the area became parched.

At least 7 sheep were carried throughout the greater part of the time of the experiments, and at one time 9 animals were grazing on the area. Since the beginning of 1930 no *Nematodirus* infected animals have been conveyed to the paddock, additions to the flock being either by natural increase or by the admission of *Nematodirus*-free individuals.

Climate of Manawatu District.

The climate in Manawatu district is temperate, in which seasons are well defined. Summer temperatures are not excessive, while in winter they do not drop to a very low level. Rainfall is distributed throughout the year, though usually in late summer and autumn it is not sufficient to prevent parching of pasture.

Species Found in Manawatu District and their Relative Numbers.

The four *Nematodirus* species found in Manawatu district were, in order of abundance of males, *N. filicollis*, *N. spathiger*, *N. abnormalis*, *N. helvetianus*. *N. filicollis* and *N. spathiger* were both abundant and were distributed throughout the district. Usually they occurred together in infections. The relative numbers varied, sometimes one and then the other being in greater numbers. Infections were found in which, while only odd individuals of *N. spathiger* occurred, several hundred of *N. filicollis* were present. The following table indicates the relative numbers of male *Nematodirus* found in infections in lambs in Manawatu district:—

Date.	*N. filicollis.*	*N. spathiger.*	*N. abnormalis.*	*N. helvetianus.*
1931				
28th July	58	31	6	—
1933				
30th November	198	11	1	—
7th December	199	1	—	—
7th ,, ...	234	—	—	—
21st ,, ...	328	4	—	—
21st ,, ...	205	126	6	—
1934				
25th January	557	1,338	23	—
25th ,, ...	233	395	5	—
1st February	1,372	757	111	—
1st ,, ...	1,296	464	22	—
9th April	160	33	10	—

Manner of Distribution of *Nematodirus* Spp. Within the Sheep.

The bulk of the data available relate to *N. filicollis* and *N. spathiger*. The smaller number of *N. abnormalis* and *N. helvetianus* present in infections and their low incidence did not enable the drawing of definite conclusions.

Small numbers of *N. filicollis* and *N. spathiger* were found occasionally in the abomasum of lambs but their presence there is to be regarded as accidental, probably having been brought about by regurgitation of food from the duodenum. The normal region of infection for all species is undoubtedly the duodenum and jejunum. In these parts the infections of each species has a characteristic distribution.

It was found that in the cases of both *N. filicollis* and *N. spathiger* the distribution of numbers along the intestine, when plotted, assumed the form of a normal frequency curve. Towards the posterior beyond the limits of the main infection, small numbers of each species occurred scattered along the remainder of the small intestine.

The areas of peak numbers of the infections of *N. filicollis* and *N. spathiger* were well defined and separate, that of the former always being anterior to that of the latter, although the infections of each species overlapped to such an extent that the areas of infection were almost the same. Infections of *N. abnormalis* overlapped with those of *N. filicollis* and *N. spathiger*, and although numbers were small in individual infections, indications were that a peak of infection occurred in the case of this species also, and that it was located posterior to that of *N. filicollis*. It was found, on the examination of infections of different ages, that the area of infection and the manner of distribution of numbers did not vary in essentials. From this it is concluded that there could have been neither migration beyond the limits of the normal area of infection nor extensive movement of individuals within the area of infection. If migration had occurred from the main area of infection the results obtained would not have been as consistent as they were, and if there had been free movement of adults within the area of infection the peak form of distribution would have disappeared, whether that movement had been active on the part of the worms or passive through transportation in food.

In view of the permanence of the arrangement of *Nematodirus* spp. in the small intestine, it is therefore inferred that the distribution of *Nematodirus* infection is determined by the early larval stages, and this view is strengthened by the fact that immature stages of *Nematodirus* were found only in the normal region of the greatest infection.

The response of individuals of any species of animal to stimuli, of intensity within normal physiological limits, is variable and in the aggregate may be expressed often in the form of a normal frequency curve.

In the case of *Nematodirus* spp. the normal frequency curve form of distribution is suggestive that the location of each species in the small intestine is governed by the time of reaction of incoming larvae to factors which they experience for the first time on entering the small intestine. It is apparent that the stimulating factors operate at the beginning of the small intestine, for adults are found close to the pyloric valve, and by similar reasoning it is seen that stimuli act over a considerable portion of the small intestine. In that incoming larvae are transported in a current of food, the time of response of *Nematodirus* larvae to stimuli causing them to take up their station in the small intestine, must be expressed in the form of a space distribution and numbers will tend to accumulate round a particular point. If this were the case it would be expected that specific differences might exist and that in mixed infections different species would have their peaks of infection separate and of consistently similar relationship to one another. This was seen to happen with *Nematodirus* spp. in which the peak of *N. spathiger* always occurred behind that of *N. filicollis*. Both *N. filicollis* and *N. spathiger* plainly are adapted to life in the same region of the small intestine, for in all cases infections overlapped completely, while each species was found in large numbers within the same host. The close proximity of the peaks, in conjunction with these facts, would seem to render it conclusive that the differences in distribution of these two species are due to specific differences in the time of reaction of larval stages to stimuli causing them to take up their station.

Nematodirus Incidence and Numbers.

It was found that the incidence of *Nematodirus* spp. varied according to the age of the sheep considered. Under normal farm conditions in Manawatu district, infection by species of this genus was confined almost entirely to lambs of less than 9 months of age. In the table following data are presented, the result of post mortem examination conducted for *Nematodirus* spp. upon 107 sheep during the period 1931–1934.

Age.	Total number of examinations.	Present in.	Absent in.
1. Less than 2 months	6	2	4
2. 3–8 months	59	59	—
3. 9–12 ,, ...	9	1	8
4. Older than 12 months	35	2	33
	109		

Although *Nematodirus* spp. could be found throughout the year in sheep, a rhythm in numbers in which there was an alternation of high numbers during November–April, and low numbers during May to October, occurred. It is the practice in this district for lambing to take place over a short period, for the most part within the months August and September, thus it comes about that the seasonal period of greatest numbers of *Nematodirus* individuals approximates to the 3–7 month stage in the life of the lamb. The rhythm in numbers is indicated in the table on page 50 which refers to total numbers of *Nematodirus* spp. found.

Factors Responsible for the Rhythm in Numbers.

In accounting for the rhythmical distribution of *Nematodirus* numbers, it was uncertain to what extent the causal factors lay without or within the sheep.

Data elucidating the causes of the rhythm were provided from the results of post mortem examinations and from series of egg counts conducted on sheep of various ages which had been maintained on an experimental paddock. The egg count record dates from November 25th, 1931, at which date counts were begun in connection with sheep Nos. 1 and 2, both ewes, 2 years of age, and upon a ewe lamb, sheep No. 3, which had been born in September 1931. The ewes presumably had recovered from Nematodirus infection before the egg counts were begun. During the course of the experiments sheep Nos. 1 and 2 remained free from *Nematodirus* spp.

In connection with sheep No. 3, infection by *Nematodirus* had already been incurred by November 25th, 1931, and eggs of this genus continued to be voided until February 18th, 1932.

On September 1st, 1932, sheep No. 1 gave birth to twin lambs (sheep Nos. 4 and 5), and sheep No. 2, on September 4th, to one lamb (sheep No. 6). In the cases of sheep Nos. 4, 5 and 6, the first examination dates on which eggs were found were November 8th, 21st, and 21st, 1932, respectively, and the final dates respectively were May 9th, 29th,

Date.	Age.	Numbers.
1931		
14–7	10 months	Nil
28–7	10 ,,	Nil
28–7	22 ,,	Few
28–7	22 ,,	91
1–8	11 ,,	Nil
2–8	11 ,,	,,
25–8	11 ,,	,,
4–9	8 ,,	4 larvae
16–9	12 ,,	Nil
19–10	5 weeks	100
23–10	5–6 weeks	Nil
23–10	5–6 ,,	,,
17–11	2½ months	,,
1932		
7–2	5–6 months	512
2–10	2 months	280
29–11	3 ,,	3,680
1933		
7–2	5 months	519
4–4	8 ,,	2,000 (over)
1–5	9 ,,	Nil
9–5	9 ,,	18
30–11	3 ,,	391
30–11	3 ,,	210 males
7–12	3–4 months	580
7–12	3–4 ,,	1,140
7–12	3–4 ,,	234 males
7–12	3–4 ,,	790
21–12	4 months	750
21–12	4 ,,	337 males
1934		
25–1	4–5 months	633 males
25–1	4–5 ,,	633 ,,
25–1	4–5 ,,	1,920 ,,
1–2	5 months	5,000
1–2	5 ,,	3,700
9–4	7 ,,	350

and 9th, 1933. No. 7 sheep, born in September 1932, was maintained free from infection until July 7th, 1933, when, at 10 months of age, it was placed in the experimental paddock. On August 7th, 1933, *Nematodirus*

eggs appeared in its faeces and after November 30th, 1933, they ceased to appear. Sheep No. 8 was born on January 22nd, 1934, and maintained free of *Nematodirus* infection until February 24th, 1934, when it was conveyed to the experimental paddock. It contracted *Nematodirus* infection, eggs being voided over the period March 23rd to July 10th, 1934. *Nematodirus* eggs, therefore, continued to be voided in the faeces of sheep 4, 5, 6, 7, 8 for the periods 182, 189, 169, 115 and 109 days. Infections in these cases consisted of two species, *N. filicollis* and *N. spathiger*.

Infectivity of Pasture at Different Times of the Year.

In the ordinary course of events the greater part of *Nematodirus* infection was incurred by lambs in the spring and early summer from September to December. It was necessary, therefore, to investigate the degree of infectivity of pasture during the period January to August.

The infections incurred by sheep Nos. 7 and 8 during February and July respectively so far as the egg counts could reveal, were of similar intensity to those acquired by each of sheep Nos. 4, 5 and 6 during the spring.

It is concluded that *Nematodirus* infective larvae are accessible to sheep in numbers of similar order throughout the year.

Course of Infection by *Nematodirus* Species.

Among lambs *Nematodirus* infection accumulates rapidly from soon after birth. Larvae in one case were found in a lamb 8 days old indicating that it was possible for infection to be incurred as soon as lambs began to nibble grass. A certain degree of regularity in the time of onset of infection in young lambs took place for in the same flock as that of the lamb to which allusion has been made above, and during the same season, 2 lambs, 3–4 weeks of age and 2 others, 5 weeks and 5–6 weeks of age, were found on post mortem examination to be free from infection.

Frequently in lambs of 2–3 months of age, *Nematodirus* spp. were in abundance, and on occasions amounted to several thousands. During the period of greatest numbers, that is, in lambs of 3–6 months of age, infections of the order of 3–4,000 worms were commonly met with. The greatest number in one individual was found in a lamb 5–6 months of age, in which 5,000 individuals occurred.

Marked dissimilarity in the intensity of infections occurred in different animals, during the period when infection was normally at its height, numbers ranging from 391 to 5,000. A decline in numbers was found to set in about the time that lambs reached 6 months of age and subsequently, about the time that they attained 9 months of age, infection had almost completely disappeared. An exceptional case was discovered in which a sheep of 22 months of age contained 91 individuals of *Nematodirus*. Although it was known to have spent its entire life on the Massey College farm as a flock animal, where it would have been exposed to infection for the whole of that period, it was uncertain to what extent the presence of *Nematodirus* was the aftermath of infection incurred early in life, or of infection re-acquired in the second year.

An indication of the length of *Nematodirus* infection in individual cases was provided by the egg counts of lambs Nos. 4, 5, 6, 7, 8. The actual length of the period of infection will be larger than that during which eggs were voided on the account of a preliminary larval stage and a concluding stage when only non-laying adults may have been present. The ages of sheep 3, 4, 5, 6, when *Nematodirus* eggs ceased to be voided were 125, 250, 270 and 246 days respectively.

Age Resistance.

The development of an age resistance to *Nematodirus* infection was found not to occur. Evidence in this connection was afforded from data secured from sheep No. 7 which had been exposed to infection for the first time when 10 months of age, in experiments on the infectivity of pasture. The development in the animal of an infection at an age when *Nematodirus* had normally disappeared, was indicative that age resistance was not developed.

The Effect of Seasonal Conditions on the Course of *Nematodirus* Infection.

In order to arrive at any definite conclusion in this respect, it would be necessary to conduct experiments involving large numbers of animals. This was not possible in the present experiments. However, a small amount of evidence bearing on the matter was available from the egg count records.

An examination of these results shows that the duration of infections of sheep 2, 3, 4, 5 and 6, incurred during the spring and summer period, differ to no appreciable extent from the durations of those incurred by sheep 7 and 8 in the autumn and the winter. It is concluded, therefore, that there was no evidence that the external conditions, by modifying the response of the sheep, were effective in causing variations in the course of *Nematodirus* infection from one season to another.

The Effect of Infection by Other Species of Nematode upon that of *Nematodirus* spp.

Nematodirus infection was found to occur in the presence, in the small intestine, of species of *Trichostrongylus colubriformis*, *T. vitrinus*, *Cooperia oncophora* and *C. curticei*. The normal distribution of the three genera *Trichostrongylus*, *Nematodirus* and *Cooperia* along the intestine shows that infection by *Nematodirus* spp. overlaps that of the other two only to a limited extent. The possibility, therefore, of any direct effect of these species on *Nematodirus* spp. is limited accordingly, and particularly would this be so in the case of *Cooperia* spp. which are located almost entirely posterior to the site of *Nematodirus* infection, rendering the latter out of range of any deleterious secretions or excretions. Furthermore, the chances of interference of *Nematodirus* spp. by *Trichostrongylus* spp. and *Cooperia* spp. being of significance, are lessened by the fact that under normal conditions of infection *Nematodirus* numbers have attained their peak and are already on the decline before those genera which are acquired during autumn and early winter reach high numbers.

There was no indication that the factors causing the elimination of other species of nematode in sheep were at the same time instrumental in removing *Nematodirus* spp. Infections by *Trichostrongylus* spp. and *Cooperia curticei* were found to extend to animals of over 12 months of age several months at least after *Nematodirus* had disappeared. *Haemonchus* in some infections, as revealed by egg counts, drew to a close before those of *Nematodirus*. *Chabertia ovina*, *Trichuris ovis*, and *Strongyloides papillosus* all followed erratic courses of infection and obviously did not have bearing on the course of *Nematodirus* infection.

Acquired Immunity.

In view of the events outlined in the foregoing, it was apparent that the only factor which could account for immunity from *Nematodirus* once

infection had been eliminated was that an acquired immunity had been developed. The results of post mortem examination and the egg counts conducted on sheep No. 3 showed that it was normally of long standing. Although the early histories of sheep Nos. 1 and 2 were not known, the fact that they remained free from *Nematodirus* may be taken as added evidence of the permanence of the acquired immunity.

From general post mortem examinations, it was apparent that the acquired immunity developed against *Nematodirus* spp. was of long standing. Occasionally odd individuals of *Nematodirus* were found in mature sheep. These cases, in view of the small numbers found, must be looked upon as exceptional and are not an indication that there had been modification of acquired immunity.

The egg count records of sheep 1 and 3 show that at various periods *Haemonchus contortus* infections were developed. In the cases of sheep Nos. 1 and 3 infection was found definitely to have been contracted through the modification of the acquired immunity previously developed against that species. However, in neither of these animals did *Nematodirus* infection develop a second time. The inference is drawn that factors which are responsible for the modification of acquired immunity to *Haemonchus* were not effective in modifying that developed against *Nematodirus*. It was found that modification of the acquired immunity to *Haemonchus* occurred during lactation in the spring and during devitalizing summer and autumn seasons.

Immunity to Superinfection.

Detailed examination of the entire population of *Nematodirus* obtained from slaughtered animals at different times showed that the rate of infection indicated by the numbers of immature stages did not proceed uniformly throughout the season.

In the following table the results of examination are shown. It will be seen that the percentages of larvae of *Nematodirus* spp. present are in most cases considerably short of the figure representing the daily average acquisition necessary to have produced the infection.

From this it is concluded that the great portion of *Nematodirus* individuals are acquired only in the life of the lamb, probably within the first 2–3 months of its life.

Further, it is concluded that the host had acquired immunity to superinfection which produced its effects on immature stages, preventing their maturity and causing their rapid elimination. From the fact that immature stages were found in small numbers within the limits of normal infections and not elsewhere, it was concluded that the immune forces became effective after the factors which determined the location of infection in the small intestine had played their part.

Date of examination.	Approximate age of lamb.	Total *Nematodirus* population.	Theoretical average rate of acquisition per day.	Immature stages found at post mortem.
7–12–33	3 months	234 males	4	3
21–12–33	3 ,,	750	12	12
21–12–33	3 ,,	337 males	6	Nil
1–2–34	5 ,,	3,680	30	1
1–2–34	5 ,,	5,000	40	130

Evidence relevant to the manner of production of immunity to superinfection was provided by the egg count results. The egg count of sheep No. 7 rose abruptly to a peak, and in this respect differed from that of sheep Nos. 4, 5 and 6. On account of it being older, sheep No. 7 would have consumed greater amounts of grass than the other lambs during the initial stage of infection, so that in the case of No. 7 numbers of *Nematodirus* must have accumulated more rapidly. The fact that a peak in the egg count was soon reached in the case of No. 7 and that its order of magnitude was not markedly different from that of the other lambs, indicates that the infection was accumulated during a shorter period than for the other lambs. This is concluded to be slight evidence that rapid accumulation of *Nematodirus* numbers results in the production of an immunity to superinfection sooner than an infection accentuated at a slower rate. It is possible that the varying numbers of *Nematodirus* spp. found in infections at the height of the season was indicative of individual differences occurring in lambs as to the amount of the requisite stimuli necessary to promote immunity to superinfection.

The Elimination of Adults.

The extent to which the acquired immunity or immunity to superinfection were effective in eliminating the mature adult population was

uncertain. It was the rule to find that infections continued for several months following the initiation of immunity to superinfection and that during that time egg-laying, the barometer of worm welfare, continued unabated.

The egg counts conducted on sheep 3, 4, 5, 6, 7, show that egg laying must have continued normally after immunity to superinfection had set in. Egg numbers in the uteri of females obtained from infections at different times of the year show that only in the later stages of infection is there a reduction in the numbers contained in individuals.

It is concluded, therefore, that immunity to superinfection is not responsible for any immediate effects on egg laying capacity of females.

In infections obtained at different times, small numbers of *Nematodirus* adults occurred scattered along the intestine beyond the main area of infection. These worms were healthy in appearance and in the case of the females, packed with eggs—and are to be regarded as having been accidentally swept from the main area of infection and on their way to the exterior. At no time have immature stages been found in the hinder part of the small intestine and it is therefore unlikely that adults could have developed in that region. The fact that isolated females should have their uterus packed with eggs would seem to indicate that they had been fertilized either in the main area and had been swept to that part or that fertilisation had taken place in the part where they were found and that the male had been swept away. Either view postulates that one or other of the sexes must have been swept along the intestine while in a healthy and functional state. This continued toll on the healthy worm population in the absence of renewal of population must be an important factor in bringing infections to a close. Occasionally, during the height of *Nematodirus* infection, females either degenerate or requiring re-fertilisation, were found in the ileum. To what extent these had been affected by an immune response was problematical.

It was found that females collected in the latter stages of infection contained smaller numbers of eggs than those in earlier stages and were obviously senile.

It is concluded, therefore, that immunity to superinfection is not effective in causing any sudden large scale alteration in adult numbers following its expression in the curtailment of the period during which infection may be acquired. There is a gradual loss of adults which from

the appearance of healthy functional activity makes it appear that the normal hazards of life in the intestine are responsible for their elimination. The extent to which the immunity response hastens senility was not clear.

From the foregoing it is apparent that immunity to superinfection, the elimination of *Nematodirus* infection usually before the host reaches 8 to 9 months of age, and the development of an acquired immunity to further infection, are all important in causing the rhythm in *Nematodirus* numbers. The practice of confining the lambing period almost entirely to August and September and the fact that there is 100% incidence in lambs of 3 to 6 months of age, cause the rhythm to be decided.

Adaptability of *Nematodirus* to Sheep and Other Considerations.

It was found that fat healthy lambs slaughtered for food purposes frequently harboured infections of *Nematodirus* of the order of 3–4,000 individuals and in one case 5,000 individuals occurred without any outward signs of infection. In no poorly conditioned lamb which had died or which had been slaughtered, were there greater number than these figures, and in no case was it conclusively shown that *Nematodirus* spp. had been the cause of death of lambs.

Under the condition of infection in these experiments, it was apparent that the early development of immunity to superinfection was instrumental in controlling numbers before they reached a level dangerous to the host.

Stoll, working with *Haemonchus contortus*, found that infection could be of three types :—

1. Fulminating and lethal.
2. Chronic and of long standing.
3. Self curative.

With *Nematodirus* self curative infections alone were found.

Sandground has come to the conclusion that parasites which are not well adapted to their hosts and cause injury, stimulate immune responses in them. Perhaps *Nematodirus* spp. under the conditions of the experiments here described, are an exception to this rule in that acquired immunity is developed, but that immunity to superinfection limits infection to a figure not injurious to the host.

REFERENCES.

BOULENGER, C. L., 1915.—"The Life History of *Nematodirus filicollis*, a Nematode Parasite of the intestine in the sheep." *Parasitology*, VII (3). (*W.L.* 16035).

GILRUTH, J. A., 1902.—"Report Division of Veterinary Science New Zealand Department of Agriculture 1901–2.

SANDGROUND, J. H., 1929.—"A consideration of the relation of host specificity of helminths and other metazoan parasites to the phenomena of age resistance and acquired immunity." *Parasitology*, 21, p. 227. (*W.L.* 16035.)

THOMSON, G. M., 1922.—The Naturalisation of Animals and Plants in New Zealand. London.

Journal of Helminthology, Vol. XIII, *No.* 2, *May*, 1935, *pp.* 59–66.

On cyst-like bodies, resembling cysts of *Heterodera schachtii* of common occurrence in British Soils.

By Marjorie J. Triffitt, D.Sc.

(*Field Officer, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine*).

In 1928 a description of the morphology of three strains of *Heterodera schachtii* attacking beet, oats and potatoes respectively, was published in the Journal of Helminthology by the present writer. This was followed in 1929 by a second publication in which the morphology of strains of the nematode attacking beet, oats, hops, mangolds and potatoes were described. In the earlier paper it was noted that cysts of the potato-strain in Hertfordshire were smaller both in average and maximum dimensions than were cysts in soil from South Lincolnshire. The Hertfordshire land had been infected by the addition of South Lincolnshire soil and the reduction in cyst-size occurred in adjacent portions of the plot to which the infection had spread during the cultivation of two successive potato crops. It was concluded that this decrease in size was a reaction to the difference in soil type, possibly associated with available oxygen.

In the second publication a further description was given of the cysts in Hertfordshire soil. In the examination of this soil was it found that although cysts containing eggs varied in size from 0·91 mm. by 0·69 mm. to 0·31 mm. by 0·17 mm., closer observation by means of a lens showed that a great number of smaller bodies similar in shape and colouration to the egg-containing cysts were also present. These small forms in fact rather exceeded the egg-containing cysts in number, and, since a perfect gradation in size was shown from the largest egg-containing cyst to the smallest egg-less form, it was assumed that all represented cysts of *H. schachtii* and they were described and figured as such. No suggestion was made as to the cause of their formation and no special significance was assigned to their presence in the soil. It was in fact thought at that time that their development was due to the change in physical conditions of the environment of the nematode attendant upon the artificial infection of a heavy clay soil by the addition of a portion of light sandy Lincolnshire soil which contained a natural infection. This seemed the more probable

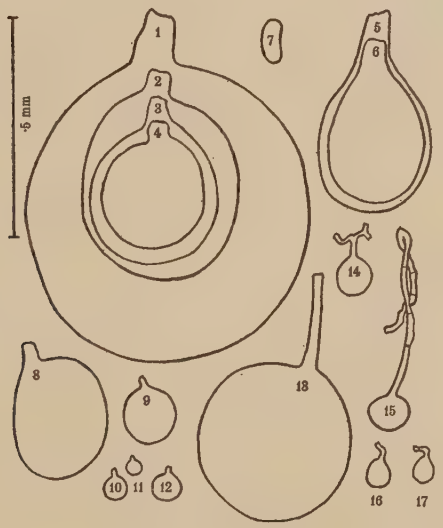
since an undoubted reduction in the size of the egg-containing cysts had occurred, and none of the small egg-less forms were present in the Lincolnshire soil.

A more detailed study of the small cyst-like bodies found in the plot of land in Hertfordshire revealed that in certain respects they differed from cysts of *H. schachtii*, and, although these differences were not marked, they were sufficiently distinct to enable counts of egg-containing cysts to be made by an experienced person without confusion arising out of the presence of the egg-less cyst-like bodies, which for laboratory purposes came to be termed " microcysts." These differences, coupled with the fact that many of the " microcysts " were actually smaller than the egg of *H. schachtii*, threw considerable doubt on their mode of origin, and the question of their identity was discussed with various scientific authorities. That they were not any stage of the life cycle of *H. schachtii*, either normal or aberrant, was finally concluded when a sample of soil was received from Mr. J. C. F. Fryer, which, although taken from land which contained no egg-bearing cysts of *H. schachtii* and on which potatoes had never been grown, yet contained great numbers of " microcysts." This view was agreed by Dr. Goffart to whom " microcysts " had been sent for examination and who published a note to that effect in a scientific paper dealing with the morphology of the various strains of *H. schachtii*.

Although no further mention of the " microcysts " has been made in the literature, it has recently appeared that certain workers have overlooked Goffart's publication, and, while continuing to hold the view that the " microcysts " represent a stage in the life-history of *H. schachtii*, attach considerable significance to their presence in soil infested by the nematode. This erroneous idea of their significance has apparently arisen out of the distribution of the " microcysts " in one district of Britain, where although they were present in great numbers in land infected with *H. schachtii* where potatoes were grown as one crop in a rotation, they were not present in other land where potatoes were grown annually. From this it was deduced that all the larvae present in a cyst possessed the vigour necessary to complete their life cycle in the season following that in which the cyst developed, but, if a suitable host plant were withheld from the land for even one year a loss of strength took place in the larvae to such an extent that although they hatched and reached the roots of the host they were unable to complete their life cycle and rounded up to form small empty cysts, the " microcysts." From an

estimation of the comparative numbers of true cysts and " microcysts " present in soil it was concluded that as many as 97 per cent. of the larvae lost vigour in this way when a short rotation of crops was practised, and since of the remaining 3 per cent. half only would be females, the annual increase in cyst-content of land under a crop rotation was comparatively small.

This theory obviously neglects one fundamental point regarding the bionomics of the nematode, namely the fact that the cyst does not normally lose all its contained larvae in any one season. This is clearly shown by examination and dissection of cysts from any soil sample, when many

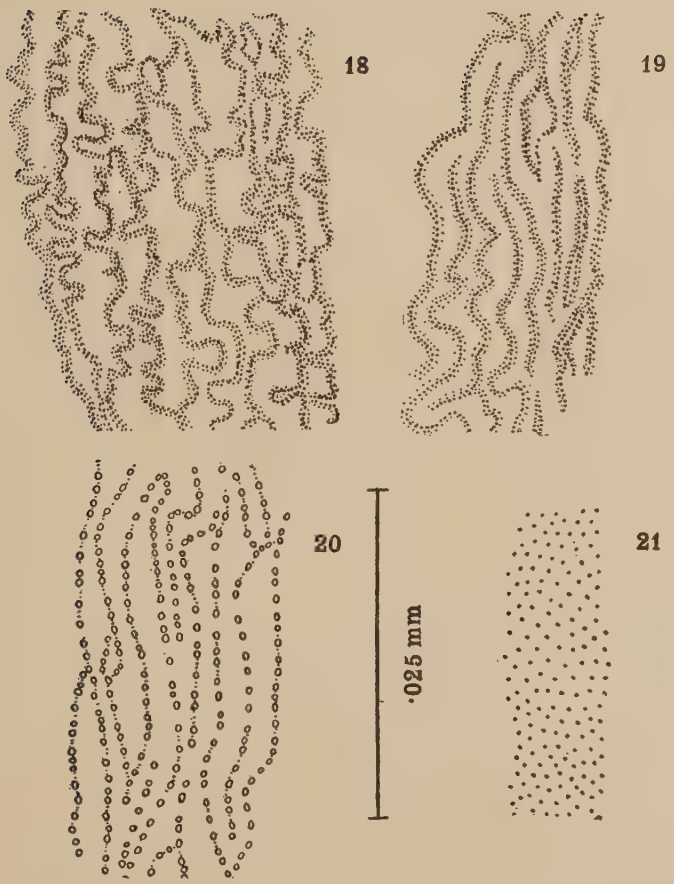


Figs. 1–4.—Spherical type cysts of *H. schachtii*, potato-strain.
Figs. 5 and 6.—Immature ovoid cysts of *H. schachtii*, potato-strain.
Fig. 7.—Egg of *H. schachtii*, potato-strain.
Figs. 8–12.—" Microcysts " with short, neck-like projections.
Figs. 13-17.—" Microcysts " with hypha-like outgrowths.

will be found to contain both embryonated eggs and empty egg membranes from which the larvae have escaped. Hence, unless it be assumed that those larvae which do not hatch on the first occasion when the host plant is available have lost the power of hatching, the larvae liberated in succeeding years would be in exactly the same state as larvae in land where a rotation of crops was practised. But it has been shown repeatedly in laboratory experiments on the hatching of the larvae from the cysts, that by the repeated application of potato root excretion all the larvae are induced to hatch in the course of a few months. This occurs irrespective of whether the cysts are specially selected as being newly formed and hence full of unhatched eggs, or are not so chosen and are therefore of different ages containing varying proportions of hatched and unhatched eggs. Further it has been recorded that cysts isolated from fields which have not grown potatoes for many years may all prove on dissection to be void of unhatched eggs. Hence it cannot be maintained that those larvae which do not hatch on the first occasion when the host plant is available have lost the power of hatching, and " microcysts " might therefore be expected to occur in land on which potatoes were grown annually.

Although this theory of the origin and significance of the " microcysts " has not been published in scientific form it has gained considerable publicity, and it therefore seems desirable to point out the morphological and other evidence which, in the writer's opinion, prove that " microcysts " are not in any way related to *H. schachtii*.

As " microcysts " were first discovered and described from Winches Farm, the Institute's Field Station, a survey was carried out to determine their distribution. Soil samples were taken from every field and plot and these were examined for " microcysts " by Morgan's flotation method. In every sample so examined " microcysts " were found although they showed considerable variation in numbers. The samples were taken from permanent pasture, arable land which had never grown potatoes and was known to be free from *H. schachtii* infection, arable land infected with various strains of *H. schachtii* and other plant-parasitic eelworms, and woodland. Detailed counts were not made but it was evident that " microcysts " occurred in the greatest abundance where heavy dressings of farm-yard manure had been applied to the arable land and in those pastures which had been heavily stocked. Whether this is of any significance or is a purely fortuitous circumstance remains unknown until the nature of the " microcysts " is determined. That they are not limited



Figs. 18-20.—Surface markings on cysts of *H. schachtii*.
Fig. 21.—Punctate markings on outer surface of " microcyst."

in distribution by available farm-yard manure is shown by their occurrence in considerable numbers in the soil of woodland and spinney. Many other soils which were available at the Institute were similarly examined and over 90 per cent. of these also contained " microcysts."

As has already been stated the " microcysts " show a very striking similarity to cysts of the potato-strain of *H. schachtii*. They are more or less spherical in form and frequently show a neck-like projection. They are, in most cases, of the same colour as true cysts, ranging from a light to a very dark brown, and, although in general they are much smaller than cysts of *H. schachtii*, the largest " microcysts " exceed in size the smallest of the true cysts.

Although immature cysts of *H. schachtii*, which tend to occur in varying proportions in all infected soils, are ovoid in shape, rounded cysts of very small size are also frequently found. These may measure as little as 0·25 mm. in length exclusive of the neck, whereas " microcysts " may occur which measure 0·4 mm. exclusive of the neck-like projection. One apparently constant distinction is, however, that the neck of the true cyst is wider, both actually and in proportion to body width, than the neck-like projection of the " microcyst." Prolonged examination of soil containing " microcysts " has shown that the neck-like projection is in fact the remains of a hypha-like outgrowth which may very rarely be found to persist in recognisable form. In some cases this extends for a considerable length without bifurcation, in others it bifurcates early ; a single individual was found with an elongated, distinctly septate outgrowth continuous with the main body but, as were all the elongated hypha-like outgrowths, of a pale colour and almost completely transparent.

The most obvious and constant differences between true cysts and microcysts lie in the details of structure of the cyst wall and the contents of the body. The true cyst contains eggs or egg membranes and, in rare cases where very newly formed immature cysts are examined, recognisable remains of the ovaries and uterus of the worm. Ninety per cent. of the " microcysts " are void of contents or contain a little granular matter only. The remainder are completely filled with what appears to be a highly granular mass of cytoplasm in which neither nucleus nor other structure can be distinguished. Although most of the " microcysts " closely resemble true cysts in colour, a few, varying in number in different samples, are of a much lighter colour and are semi-transparent.

These light coloured forms show a great range of size and there seems no reason for supposing that they represent a distinct organism as in other respects they seem to be identical with the darker forms. Other differences between the wall or integument of true cysts and " microcysts " can be seen when they are examined together by reflected light, for whereas the wall of the true cyst is of a rather dull surface, the wall of the " microcyst " usually appears smooth and shiny and reflects the light. The reason for this is revealed by examination under a high power of the microscope, for, whereas the outer surface of the integument of the " microcysts " is smooth or minutely punctate, the surface of the cyst wall is constantly sculptured. The sculpturing is linear in form and shows a series of roughly parallel convoluted lines, the degree of convolution varying greatly from cyst to cyst. Occasionally, but rarely, the lines are marked by serial pittings in the surface, but this pitting is of linear arrangement and quite distinct from the punctate markings on the microcysts.

One further difference between true cysts and " microcysts " becomes clearly evident during dissections. When a true cyst is subjected to pressure the cyst wall is found to bend and give readily, more especially when the cyst is not completely filled with embryonated eggs, but when pressure is brought to bear upon a " microcyst " it is found to be hard and rigid and to crack rather than bend, showing a distinctly laminated structure when the broken edges are examined.

Although the systematic position of the " microcysts " remains obscure, from their distribution in soils, and the numerous morphological distinctions between them and the cysts of the nematode, it seems clear that they do not represent any stage of the life cycle of *Heterodera schachtii*, either normal or aberrant and therefore they should be disregarded in assessing the nematode population of soils. The " microcyst " wall gives a negative reaction to chemical tests for cellulose and, according to Goffart, a positive reaction for chitin. Goffart makes a tentative suggestion that they may represent a stage in the life cycle of *Heterodera marioni* but this parasite is of very rare occurrence in Britain outside glass-houses, and Goffart has not observed any forms with hypha-like outgrowths.

References.

Goffart, H., 1934.—" Die Bestimmung von Rüben-, Hafer- und Kartoffelnematoden auf grund von Bodenuntersuchungen." *Z. PflKrankh.*, Bd xliv, pp. 36–41. (*W.L.* 23540b.)

Triffitt, M. J., 1928.—" On the Morphology of *Heterodera schachtii* with Special Reference to the Potato-strain." *J. Helminth.*, vol. vi, pp. 39–50. (*W.L.* 11224b.)

——, 1929.—" Further Observations on the Morphology of *Heterodera schachtii*, with Remarks on the Bionomics of a Strain attacking Mangolds in Britain." *J. Helminth.*, vol. vii, pp. 119–140. (*W.L.* 11224b.)

Journal of Helminthology, Vol. XIII, *No.* 2, *May*, 1935, *pp.* 67–76.

Some Observations on *Necator suillus* Ackert and Payne, 1922.

By J. J. C. Buckley, D.Sc.

(*Wandsworth Research Scholar, London School of Hygiene and Tropical Medicine.*

Introduction.

Since the publication of Goodey's (1923) experimental work with *Necator americanus* and the domestic pig, there have been but brief references in literature to the species *Necator suillus*, which, since its erection in 1922 by Ackert and Payne, has been questioned as to its validity by more than one authority. The position with regard to this vexed species and the previous literature appertaining to it was aptly summarised in the first-mentioned work, so that it is unnecessary here to do more than state briefly the main aspects of the case, which are, that Necators found in pigs in Trinidad, B.W.I., by Ackert and Payne, were described by them as morphologically distinct from the human *Necator americanus*, and were named *N. suillus*. Subsequently, Gordon (1923), who had previously (1922) identified Necators from pigs in Amazonas as *N. americanus*, described this pig material with special reference to the points of differentiation set out in the original description of *N. suillus*, and concluded, in effect, that the latter was not a valid species. This finding has been endorsed by Lane (1932) and apparently also by Yorke and Maplestone (1926). Although Goodey's work leads to the conclusion that the human and porcine Necators are at least biologically distinct, no protagonist of the morphologically separate identity of the porcine Necator has been forthcoming since it was originally described as a new species. In view of the somewhat overpowering opinions extant against its validity, it is with some diffidence that the writer ventures to reopen a question which has been apparently closed for so many years. It was, in fact, reopened fairly recently by the writer (1933) in an article describing an experimental human infection with Necator from pigs. In this article, the material used was referred to as *N. suillus*, not as a convenient method

of indicating its source, but from a sound conviction that the species was a perfectly valid one. Somewhat arbitrarily, perhaps, no reasons were given at the time for this opinion, but a previous thorough comparative examination of both human and porcine Necators having convinced the writer that the species *N. suillus* should never have been questioned in the first instance, it was not considered necessary to recapitulate or amplify Ackert and Payne's original adequate description. This omission evoked the following criticism :—" The assumption underlying the acceptance by helminthologists of *N. suillus* as a morphological species seems to depend on reported differences of a grade which the anthropologist does not accept as sufficient to exclude the Hebrew, the ' Aryan ' or the Australian aborigine from the species *Homo sapiens* " (Clayton Lane, *Trop. Dis. Bull.*, xxx, 10, p. 693) which prompts the writer to set down the results of personal observations on the morphology of the species in question. It will be noted that the following opinions are merely those of a helminthologist. No appeal is made, for example, to the conchologist, whose criteria of specific morphological diagnosis, as exemplified in genera such as Planorbis, would perhaps unduly weight the balance in favour of the validity of *N. suillus*, if applied in this case.

Morphology of *N. suillus*.

In his paper claiming the invalidity of *N. suillus*, Gordon examined the individual points of differentiation as set out by Ackert and Payne, with reference to his Amazonas and Jamaica material. With none of these is he in agreement. Admittedly some of them are rather minute and one is constrained to make allowances for the personal factor in discrepancies between two workers in their interpretation of the significance of a diagnostic feature. With regard to others, however, such as the comparative length of the spicules, there ought to be no difference of opinion. Against this particular feature, one of the most salient which have been observed, Gordon's argument is most unconvincing. Although his measurements of the spicules in the two forms give the striking figures of 0·38 to 0·6 mm. in the pig Necator as against 0·82 to 0·98 mm. in the human form, yet he endeavours to deprecate their undoubted importance as a diagnostic feature by the discovery that the ratio of length of spicule to length of body is not constantly different in the two forms. In the

following section on the statistical treatment of certain measurements, it will be seen that if this ratio is calculated for a number of specimens, the average ratio *is* significantly different in the pig and human Necators.

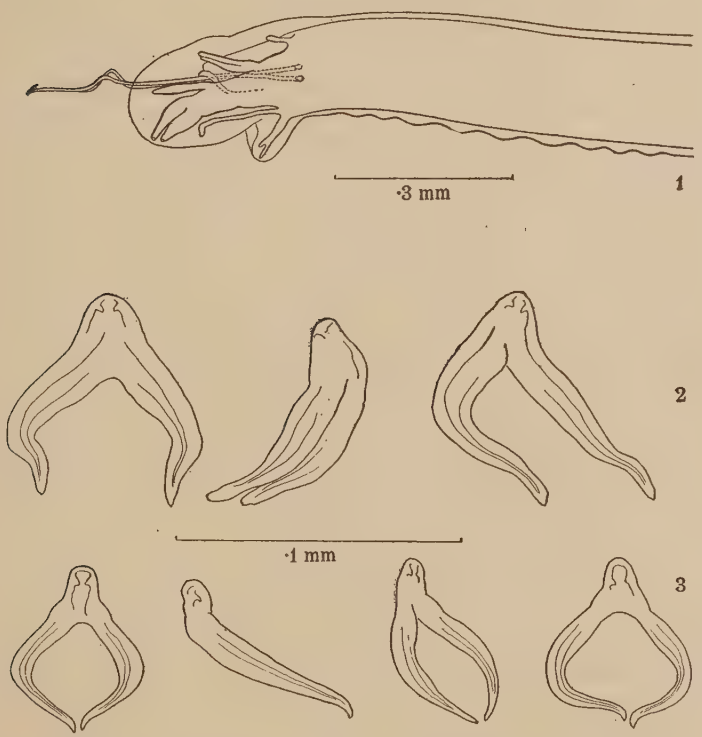


Fig. 1—Posterior extremity of male *N. suillus* showing dorsal cuticular ridges, bursa and spicules. Fig. 2—Three telamons of *N. americanus*, viewed from different angles. Fig. 3—Four telamons of *N. suillus*, viewed from different angles.

This fact, in conjunction with the remarkable difference in spicule length, should be sufficient to exclude entirely any notion of identity between the two species. The validity of *N. suillus*, however, is by no means exclusively dependent on these two points. The writer has studied ample

material collected from pigs slaughtered in Port-of-Spain and has compared it with human Necators from St. Joseph's Hospital, Trinidad, B.W.I., which were kindly provided by Dr. Innis. The writer has no hesitation in endorsing many of the less striking points of difference enumerated by Ackert and Payne, in particular those in connection with the oral papillae, the mouth armature and the dorsal bursal rays. Some further differential characters were also found in this Trinidad material which serve to strengthen the validity of *N. suillus*. It was found that the structure of the telamon showed constant differences in the pig and human Necators. The telamon in *Necator* is a chitinous organ situated at the tip of the male genital cone, and appears to function partly as an accessory piece. Roughly resembling a horse-shoe in shape, it forms a rim about the distal extremity of the cone, which is usually truncated dorso-ventrally at an angle of about 45° to the longitudinal axis of the body. At the dorsal side of the cone the two " prongs " of the telamon approach one another but do not meet, while on the ventral side the telamon projects posteriorly as a grooved process through which the spicules run. The difference in the size of the telamon in the two species is well marked and is illustrated in Figs. 2 and 3. The difference in shape is even more characteristic. Thus, in *N. suillus* the tips of the " prongs " approach very closely and in some cases are almost in contact. From their origin at the ventral grooved process the " prongs " taper gradually almost to a point at their tips, which are slightly splayed. In *N. americanus* on the other hand the tips are widely separated, due to the fact that the splaying begins much farther back from their distal extremities (Fig. 2). A second morphological difference was noted in the cuticle on the dorsal surface of the body just anterior to the bursa in *N. suillus*. The cuticle in this region is raised into a number of transverse ridges or corrugations which run half-way around the body, and have a maximum height along the dorsal line. From about seven to twelve in number they tend to decrease gradually in size anteriorly and posteriorly, and the general appearance is suggestive of the normal wrinkling of the cuticle of a nematode which occurs with any exaggerated flexure. Since, however, these ridges are present in every worm, irrespective of whether the posterior part of the body is bent dorsally or ventrally (Fig. 1), it is evident that some more deep-seated cause must be sought. It may be suggested that while the male is *in copula* with the female, its body is sharply bent dorsally in the tail region, and that the cuticle at this time being still somewhat plastic,

retains the ridges permanently by means of a subsequent loss of plasticity. Again, in the spicules of *N. suillus* a curious feature was noted. At a distance from their distal ends, equal to about a quarter the total length of the spicules, there is a definite " kink," which is especially noticeable in partly extruded spicules (Fig. 1). It is possible that this feature, like the previous one, is also an acquired character, and has been brought about during insertion of the spicules into the female genitalia. It is significant, however, that both these features are constantly present in this material but were not seen in *N. americanus*.

Statistical Treatment of Measurements of *N. suillus* and *N. americanus*

The material consists of a number of measurements of males and females of *N. suillus* and *N. americanus*. In the case of the females, the body length (L), the tail length (T) and the ratio L/T were found for 30 specimens of each species, giving six samples in all. In the case of the males, which had been cleared in beechwood creosote, the body length, the spicule (S) and the ratio L/S were found for 50 specimens of *N. suillus* and 25 specimens of *N. americanus*. In Table I are set out the mean of these measurements with the standard error, the standard deviation (σ) and the coefficient of variability (V).

The comparisons of pairs of samples, set out in Table II, give the difference between the two means with the standard error of the difference, the ratio of the difference to its standard error and the value t, obtained by applying Fisher's t-test. If two means are $M_1 \pm a$ and $M_2 \pm b$, then the difference is given by $(M_1 - M_2) \pm \sqrt{a^2 + b^2}$, which may be put as $M_3 \pm c$. Then the ratio M_3/c is such that values of two and over indicate a probability that the two samples do not come from the same population, *i.e.*, that the difference is significant. The value t, which is comparable in magnitude with the ratio M_3/c, gives a more accurate measure of significance, particularly with small samples.

The comparison of the 25 *N. americanus* males with the 50 *N. suillus* males shows highly significant differences between the two species, not only in body length and spicule length, but also in the ratio L/S. The

view that *N. americanus* might parasitize the pig and be abnormally small in that host can be maintained only with difficulty in the face of such a significant difference in the ratios. The coefficients of variability are of some interest here as they show that in *N. suillus* and *N. americanus* the

Table I.

Sample	Description of sample		Mean	σ	V. %	No. of variates
A_1	*N. americanus* ♂	Body length L	7·716 ±0·0979 mm.	0·4895 mm.	6·345	25
A_2	,,	Spicule length S	0·9388±0·00839 mm.	0·04195 mm.	4·47	25
A_3	,,	L/S	8·212±0·1095	0·5477	6·67	25
B_1	*N. suillus* ♂	Body length L	5·926±0·0473 mm.	0·3347 mm.	5·65	50
B_2	,,	Spicule length S	0·4322±0·00322 mm.	0·0228 mm.	5·28	50
B_3	,,	L/S	13·692±0·1638	1·158	8·46	50
C_1	*N. americanus* ♀	Body length L	11·15±0·1433 mm.	0·785 mm.	7·04	30
C_2	,,	Tail length T	0·1923±0·00365 mm.	0·02 mm.	10·4	30
C_3	,,	L/T	58·6±1·3635	7·497	12·76	30
D_1	*N. suillus* ♀	Body length L	7·553±0·1315 mm.	0·7202 mm.	9·53	30
D_2	,,	Tail length T	0·193±0·00414 mm.	0·0227 mm.	11·75	30
D_3	,,	L/T	39·53±0·7527	4·123	10·43	30

spicule length shows the least scatter from the mean, and the ratio L/S the greatest scatter. Thus, whilst the ratio L/S is of value in demonstrating the separate identity of *N. suillus*, in actual diagnosis the simplest and most reliable criterion would be the length of the spicules. The comparison of the males of the two species also shows that the difference in spicule length has a very high degree of significance—the highest in the present series.

In the case of the females the body length of *N. suillus* shows a highly significant difference from that of *N. americanus*, but the tail lengths are the same in the two species. Hence the ratio L/T also shows a significant difference. The coefficients of variability and the estimates of significance both indicate that the body length is the useful diagnostic character ;

TABLE II.

Samples compared	$(M_1-M_2)\pm$S.E.	$\frac{M_1-M_2}{S.E.}$	t
A_1-B_1	1·79 ±0·1087 mm.	16·467	18·34
A_2-B_2	0·5066±0·00899 mm.	56·35	66·79
A_3-B_3	5·48 ±0·197	27·82	22·146
C_1-D_1	3·597 ±0·1945 mm.	18·49	18·18
C_2-D_2	0·0007±0·0055 mm.	—	0·125
C_3-D_3	19·07 ±1·558	12·24	12·02

it shows less variability in both species than either the tail length or L/T ratio, and of these three characteristics it gives the highest significance in a comparison of the two species.

The foregoing statistical analysis of the data seemed desirable in the present case where the validity of various measurements was in question. But that is not to say that routine diagnosis must make use of complex statistical manipulations. On the contrary, this analysis has shown that, on the basis of the present material, the females, assuming that they are fully-grown, could be distinguished by a simple measurement of body length, and the males simply by a measurement of the spicules.

Biological Relationships.

The consideration of the biological relationships between the porcine and the human Necators is based on (*a*) natural infections and (*b*) experimental infections. (*a*) If, as it has been suggested, the porcine Necator is

in fact *N. americanus*, which has infected pigs accidentally, and is to be regarded merely as an occasional parasite in this host, then one would expect to find the worms very rarely in pigs, and in small numbers. The writer's experience bears out neither of these conditions, for a very high percentage of pigs examined in Trinidad was found infected on autopsy, and in many of these the infection was a very heavy one—in one instance over 700 adult Necators were taken from a single animal. It cannot therefore be argued that the pig is not well adapted as a host for Necator. (*b*) If the porcine Necator is *N. americanus*, then in view of the high percentage of pigs which are found naturally infected with it and in view of the heavy infections with it which occur, it should be a relatively easy matter to infect pigs experimentally with *N. americanus* from a human source. Goodey's experiments have shown this to be extremely difficult —probably impossible. Gordon's attempts on the same lines gave inconclusive results and Ackert and Payne's negative results have not been accepted by some authorities because the pigs used in their experiments were *post mortem* found to be infected with Necators identified by them as *N. suillus*. On the bases of Goodey's results therefore it may be concluded that Necators which occur as natural infections in pigs have arrived there from a porcine source, and therefore that the porcine Necator is a strain distinct from the human Necator and is well adapted to a porcine host. Morphological reasons have already been given to show that the porcine Necator is not merely an undersized *N. americanus*, and the experimental data bear them out. There are, however, some further experimental data to support this argument. Lane (1932) in dismissing *N. suillus* as an invalid species, expressed the opposite view as follows :— " The bodily shape of the parasite varies with the method and time of fixation, and its size with the host parasitized, just as *A. duodenale* in the tiger is smaller than in man." Now, if the porcine Necator is in reality *N. americanus* which owes its small dimensions merely to the fact that it is inhabiting an abnormal host then it is reasonable to expect that if it is returned to the normal human host, it will reassume the normal dimensions of *N. americanus*. The writer's (1933) experimental human infection, in which the infective larvae used were obtained by culturing uterine eggs of pig Necators in sterilised pig's faeces and sterilised soil, fulfils these conditions, and it was found that the adult Necators subsequently recovered by means of an anthelmintic had *not* assumed the dimensions of *N.*

americanus, but retained the small dimensions both of body and spicule length which are so characteristic of *N. suillus*.

Summary.

From morphological, biological and experimental data it is concluded that the attempt to merge *Necator suillus* in the synonymy of *N. americanus* is utterly unjustified.

Acknowledgment.

The material forming the basis of this paper was studied during tenure of a Wandsworth Scholarship from the London School of Hygiene and Tropical Medicine at the Imperial College of Tropical Agriculture, Trinidad. The writer wishes to acknowledge his indebtedness to the Principal and Staff of the Imperial College for their help and kindness whilst occupying a laboratory there through the courtesy of the Governing Body. Thanks are also due to Professor R. T. Leiper for some useful suggestions and to Dr. B. G. Peters for very material assistance in the section on statistical calculations.

References.

Ackert, J. E. & Payne, F. K., 1922.—" Investigations on the Control of Hookworm Disease. V. The Domestic Pig and Hookworm Dissemination." *Amer. J. Hyg.*, II, No. 1, pp. 39–50. (*W.L.* 600a.)

——, 1923.—" Investigations on the Control of Hookworm Disease. XII. Studies on the Occurrence, Distribution and Morphology of *Necator suillus*, including Descriptions of the other species of Necator." *Amer. J. Hyg.*, III, No. 1, pp. 1–25. (*W.L.* 600a.)

Buckley, J. J. C., 1933.—" *Necator suillus* as a Human Infection." *Brit. med. J.*, pp. 699–700. (*W.L.* 3579.)

Goodey, T., 1923.—" *Necator americanus* and the Domestic Pig." *J. Helminth.*, I, pp. 161–164. (*W.L.* 11224b.)

GORDON, R. M., 1922.—" The Occurrence of Ankylostomes resembling *Necator americanus* amongst domestic pigs in Amazonas." *Ann. trop. Med. Parasit.*, XVI, No. 3, p. 295. (*W.L.* 1063.)

——, 1923.—" A further note on the Occurrence of Ankylostomes resembling *Necator americanus* amongst domestic pigs in Amazonas." *Ann. trop. Med. Parasit.*, XVII, No. 2, pp. 289–298. (*W.L.* 1063.)

LANE, C., 1932.—" Hookworm Infection." Oxford University Press, 319 pp.

YORKE, W. & MAPLESTONE, P. A., 1926.—" The Nematode parasites of Vertebrates." xi+536 pp.

Journal of Helminthology, Vol. XIII, *No.* 2, *May*, 1935, *pp.* 77–90.

Potato Eelworm (*Heterodera schachtii*) Investigations.

By J. Carroll, M.Sc., D.I.C., A.R.C.Sc.I., N.D.A., and
E. McMahon, M.Sc., B.Agr.Sc.

(*Agricultural Zoology Department, University College, Dublin.*)

A previous paper by Carroll in 1933 described the results of research on the potato eelworm in the Irish Free State up to the year 1932. The table on p. 145 of that paper sets forth the four series of pot experiments which were carried out in 1932 and shows the degree to which the various pots in the different series were artificially infected (before planting) with eelworm cysts for the purpose of the experiments. In assessing the degree of potato sickness which developed in these pots and outlining the results of the experiments, reference was made to the following points :—appearance and size of the overground parts of the plants ; degree of development and nature of the root system ; presence or absence of different fungus diseases ; abundance of eelworm cysts on the roots (as far as could be judged by a visual examination) ; weight of tubers produced. Before the paper went to press, the actual cyst content of the soil in the different pots at the end of the growing season had not been determined. That has since been done and the figures are presented in Table I on the following page.

From the table it is quite obvious that a correlationship exists between the number of eelworm cysts present in the soil and the possible increase (or decrease) in number which may result from the growing of a potato crop. When sterilized soil (pots 17 to 22) and soil naturally free from eelworm (pots 25 to 30) were artificially infected with eelworm cysts the increase in the number of cysts as a result of growing a potato crop was more or less inversely proportional to the number of cysts originally added. The figure obtained for pots 11 and 12 (sieved soil) is also in conformity with the counts in series III and IV From the counts obtained it would appear that, under the conditions obtaining in the experiments, soil with an initial content of about 5 or 6 cysts per c.c. would still have about the same number after growing potatoes. In soil which had a natural infection of 2 cysts per c.c. (pots 13 and 14) the number was doubled by growing potatoes, while in soil which had a natural infection of 5 cysts per c.c. (pots 1 and 2) the number of cysts

Table I.

Serial No. of Pots.	Cyst content of soil before planting.	Cyst content of soil after growth of plant.	Change in cyst content.
Series I (originally infected)			
Pots 1 and 2	5 per c.c. (natural)	3·6 per c.c.	28% decrease
,, 3 ,, 4	0 (sterilized)	0 ,,	—
,, 5 ,, 6	3 per cc. (added after sterilization)	13 ,,	333% increase
Series II (originally infected)			
Pots 1 and 2 (same as Series I)			
Pots 7 and 8	0 (sieved)	practically nil	—
,, 9 ,, 10	0 (sieved and sterilized)	0	—
,, 11 ,, 12	3 per c.c. (added after sieving)	6·8 per cc.	126% increase
Series III (originally infected)			
Pots 13 and 14	2 per c.c. (natural)	4 per c.c.	100% increase
,, 15 ,, 16	0 (sterilized)	0	—
,, 17 ,, 18	1 per 4 c.c. (added after sterilization)	3·5 per c.c.	1,300% increase
,, 19 ,, 20	1 per c.c. (,, ,,)	7·5 ,,	650% ,,
,, 21 ,, 22	4 ,, (,, ,,)	15 ,,	275% ,,
Series IV (originally free from eelworm)			
Pots 23 and 24	0 (natural)	0	—
,, 25 ,, 26	1 per 4 cc. (added)	3·5 per c.c.	1,300% increase
,, 27 ,, 28	1 per cc. (,,)	5·6 ,,	460% ,,
,, 29 ,, 30	4 per cc. (,,)	6·9 ,,	72% ,,

present after the growth of potatoes was actually less. It is obvious that when the number of eelworm cysts in soil, preparatory to growing potatoes, is high, the mortality among the young eelworms either before entry to potato roots or in the roots is very much higher than when the initial number of eelworm cysts in the soil is small. The explanation for this undoubtedly is that the potato roots available can only maintain a limited number of eelworms (irrespective of the number which hatch out), and the ratio existing between the number which actually hatch and those which eventually reach maturity determines the relationship between the cyst content of the soil before and after growing a potato crop.

During the last few years it has been the writers' experience that the cyst content of potato sick soil, which is allowed to grow potatoes for a few years in succession, generally remains fairly constant in the region of about 4 to 6 cysts per c.c. Starting with a soil having about 1 or 2 cysts per c.c. it has generally been found that after growing one potato crop the cyst content rises to about the 4 to 6 figure and afterwards remains fairly steady. On the other hand if the soil has an initially high cyst content (say about 10 to 12 cysts per c.c.) the number generally becomes much reduced before a fairly constant figure is reached.

The writers have also found (as is described later) that there is a definite relationship between the cyst content of the soil (meaning cysts containing viable eggs) and the intensity of potato sickness in all cases where the cyst content of the soil is not greater than about 3 or 4 per c.c. It has moreover been observed that about 4 cysts per c.c. can give rise to potato sickness in its most severe form and accordingly there cannot be a relationship between the cyst content of soil and intensity of disease when the cyst content rises above 4 per c.c.

Series II and IV of the 1932 experiments (previous paper) made two points appear obvious, namely, that the development of potato sickness was definitely caused by the presence of potato eelworm in the soil and that the intensity of the sickness was determined by the cyst content of the soil (before planting). It was considered advisable to repeat in 1933 the series II and IV experiments of 1932. These 1933 pot experiments were therefore designated Series II A (using naturally potato sick soil for sieving) and Series IV A (using soil which had never previously grown potatoes and was therefore free from eelworm). All the details

of procedure in carrying out these pot experiments were exactly the same as those already described for the 1932 experiments. The following table shows the treatment of the various pots.

TABLE II

Series.	Serial No. of pots.	Treatment of soil used.
II A (Using naturally potato sick soil)	40 and 41	Untreated (as obtained from field).
	42 ,, 43	Soil sieved through a sieve containing 100 wires to the inch and fine portion used for experiment.
	44 ,, 45	Sieved (same as 42 and 43) and afterwards sterilized.
	46 ,, 47	Sieved (same as 42 and 43) and afterwards infected with cysts at the rate of 1 *cyst* for *each c.c.* of soil in pot.
	48 ,, 49	Sieved (same as 42 and 43) and afterwards infected with cysts at the rate of 4 *cysts* for *each c.c.* of soil in pot.
IV A (Using soil that had never previously grown potatoes)	50 and 51	Untreated (as obtained from field).
	52 ,, 53	Steam sterilized.
	54 ,, 55	Steam sterilized in March and afterwards infected with cysts at the rate of 4 *cysts* for *each c.c.* of soil in pot.
	56 ,, 57	Untreated but infected with cysts at the rate of 1 *cyst* for *each c.c.* of soil in pot.
	58 ,, 59	Untreated but infected with cysts at the rate of 2 *cysts* for *each c.c.* of soil in pot.
	60 ,, 61	Untreated but infected with cysts at the rate of 4 *cysts* for *each c.c.* of soil in pot.

The results obtained were in exact conformity with the 1932 results. On the roots of most of the plants, except those growing in sterilized soil (44, 45, 52, 53, 54 and 55), a certain amount of *Rhizoctonia solani* and *Colletotrichum atramentarium* was present but in no case abundant. The degree of root decay and also the abundance of eelworm cysts when present on

the roots can best be described by saying that both were very closely in keeping with the appearance of the overground parts of the plants.

The outstanding fact from Series II A is that sieving (in such a manner as to remove cysts completely) removes the cause of potato sickness and that the addition of cysts to soil which has been freed from them by sieving causes the disease to reappear in intensity proportional to the number of cysts added. It is significant to note that the plants in pots 44 and 45 (sterilized after sieving) were in no way better than the plants growing in soil which was sieved only (42 and 43).

The outstanding fact from Series IV A is that the addition of cysts to soil which has never previously had eelworm infection causes the appearance of the typical potato sickness symptoms in intensity proportional to the number of cysts added.

As has already been stated it is not claimed that a relationship exists between cyst content of soil and intensity of potato sickness when the cyst content of the soil is over about 4 per c.c.

In Series III of the 1932 experiments (previous paper) varying quantities of eelworm cysts were added to different pots of soil which had been sterilized a short time previously. Potato plants were then grown in these pots and it was found that obvious symptoms of potato sickness did not appear, even in the pots to which 4 cysts per c.c. of soil had been added. Yet, when the roots of the plants were examined about the middle of July cysts were found on them in numbers apparently proportional to the numbers of cysts originally added.

The soil from each of the pots in this series was stored in its own pot so that potatoes could be again planted in it the following spring. During the winter the cyst content of the soil in each pot was determined and the figures obtained have already been given on page 78 (2) of this paper. From these figures it will be seen that the cyst content of the soil in each pot increased very considerably in spite of the fact that obvious symptoms of potato sickness did not show up in any of the pots.

In the spring of 1933 half a pound of well rotted sterilized leaf mould and 3 grms. of mixed artificial fertilizer were added to each pot which comprised Series III of the 1932 experiments and a sprouted tuber of certified " Up-to-Date " " seed " was then planted in each pot.

As growth proceeded it soon became apparent that the plants in all the pots containing eelworm cysts were suffering badly from potato sickness.

There was scarcely any difference in appearance or yield between the plants in the soil which was sterilized and infected with varying amounts of cysts the previous year and the plants in the control pots of unsterilized soil. The plants in the soil which was sterilized the previous year but not afterwards infected with cysts were very good.

The results of these experiments were in conformity with the results obtained in England by Buckhurst and Fryer (1931) and left unsolved the problem why soil, which has been sterilized and then infected with eelworm cysts, can grow, in the first year, a good crop of potatoes showing little or no sign of potato sickness. The fact that such could happen has been accountable for many suggestions that potato eelworm is not the cause of the well-known potato sickness, or at least that it is only one of a number of contributory causes.

1934 Experiments.

On giving consideration to the problem mentioned in the foregoing paragraph the idea suggested itself that the sterilization of soil might have some influence on the hatching of eelworm eggs subsequently added (in cysts), and further that such influence might become less with the lapse of time after sterilization. Bearing these points in mind preparations were made to lay out the following series of experiments :—

Series A : Laboratory experiment designed to test the rate of hatching of eelworm eggs in leaching from unsterilized soil growing potatoes compared with the rate of hatching in leachings from soil sterilized at different times and growing potatoes.

Series B : Pot experiment in which potatoes are grown in soil sterilized at different times before planting and to which known quantities of cysts are added at planting time.

Series C : Pot experiment in which eelworm larvae, hatching out in the laboratory from known quantities of cysts, are watered into pots containing potatoes growing in recently sterilized potato sick soil.

Series D : Same as Series C but potatoes growing in soil naturally free from eelworm (soil which had never previously grown potatoes).

Series E : Pot experiment in which eelworm cysts are added to recently sterilized soil and as growth of potato plant in soil proceeds the soil is

watered with dilute potato root extract (leaching from unsterilized soil growing potatoes) instead of water.

During the autumn of 1933 a quantity of soil with a natural infection of potato eelworm (3·2 cysts per c.c.) was obtained and divided into a number of lots for the following purposes :—

Lot 1. To be left unsterilized.
,, 2. To be sterilized about 15th September.
,, 3. ,, ,, 1st November.
,, 4. ,, ,, 15th December.
,, 5. ,, ,, 1st February.
,, 6. ,, ,, 4th April.

On the dates indicated sterilization was carried out in an autoclave and immediately afterwards the soil was placed in a large box out of doors (except lot 6 which was immediately potted up). During the interval between sterilization and potting time (first week of April) the soil in the boxes was shovelled up once a week so that it might become well weathered.

For the purpose of the hatching experiments in the laboratory (Series A) it was decided to collect cysts directly from the roots of potatoes and not to depend upon cysts obtained from soil. Accordingly, a large number of cysts were taken from the roots of the 1933 pot plants and in order that the cysts used might be of as uniform a size as possible the largest were selected.

For the pot experiments it was necessary to collect a very great quantity of eelworm cysts. This was done during the winter by floating off cysts from eelworm infected soil and afterwards separating the cysts from the mass of material floated off by the process described in the previous paper. This was an exceedingly tedious undertaking but eventually almost one-third of a million cysts free from contaminating material were collected. This quantity of cysts weighed about 6 grms. (about 2,600 cysts per ·05 grm.). The weight of cysts required for each pot was calculated and weighed out.

Table III explains how the pots were arranged in the different series.

Potting was done during the first week of April, all the details as regards the operation being exactly the same as described on p. 147 of previous paper.

" Seed " tubers of variety " Up-to-Date," certified free from leaf roll and mosaic diseases, were used and on April 10th a sprouted tuber was

planted in each pot. All the tubers used were about the same size and each generally had three sprouts. After planting the pots were sunk in the soil out of doors and during the growing period were watered as often as considered necessary.

TABLE III.

Series.	Serial No. of pots.	Treatment.
B—Using potato sick soil	101 and 102	Untreated (as obtained from field).
	103 ,, 104	Sterilized Sept. 15th—Infected at 1 cyst per c.c. of soil.
	105 ,, 106	,, ,, 15th— ,, ,, 3 ,, ,, ,,
	107 ,, 108	,, Nov. 1st— ,, ,, 1 ,, ,, ,,
	109 ,, 110	,, ,, 1st— ,, ,, 3 ,, ,, ,,
	111 ,, 112	,, Dec. 15th— ,, ,, 1 ,, ,, ,,
	113 ,, 114	,, ,, 15th— ,, ,, 3 ,, ,, ,,
	115 ,, 116	,, Feb. 1st— ,, ,, 1 ,, ,, ,,
	117 ,, 118	,, ,, 1st— ,, ,, 3 ,, ,, ,,
	119 ,, 120	,, Apr. 4th— ,, ,, 1 ,, ,, ,,
	121 ,, 122	,, ,, 4th— ,, ,, 3 ,, ,, ,,
	123 ,, 124	,, ,, 4th—Not infected with cysts afterwards
C—Using potato sick soil recently sterilized (Apr. 4th)	101 and 102	Untreated (same pots as Series B).
	123 ,, 124	Sterilized only (same pots as Series B).
	125 ,, 126	From time potatoes appear, larvae, hatching out in lab. from 4,000 cysts (1 per c.c. of soil) watered in.
	127 ,, 128	Same as 125 and 126 but larvae from 12,000 cysts (3 per c.c. of soil) used.
	129 ,, 130	Potatoes allowed to become well grown (about 4 ins. high) and afterwards larvae hatching from 4,000 cysts watered in.
	131 ,, 132	Same as 129 and 130 but larvae from 12,000 cysts.
D—Using soil that had never previously grown potatoes	133 and 134	Untreated (as obtained from field).
	135 ,, 136	Untreated, but from time potatoes appear larvae hatching from 4,000 cysts watered in.
	137 ,, 138	Same as 135 and 136 but larvae from 12,000 cysts.
	139 ,, 140	Untreated, potatoes allowed to become well grown and afterwards larvae hatching from 4,000 cysts watered in.
	141 ,, 142	Same as 139 and 140 but larvae from 12,000 cysts.
E—Using potato sick soil	143	Sterilized April 4th, then infected with cysts at rate of 3 per c.c. of soil and as growth proceeded plant watered with potato root extract.

The larvae required for watering into the pots in Series C and D were hatched out in the laboratory. The procedure as regards hatching and handling was as follows :—At the appropriate time (about a week before the larvae were needed) the various weighed lots of 4,000 and 12,000 cysts

were placed in small beakers, and potato root extract (leaching from unsterilized soil growing potatoes) added. The beakers were kept in an incubator at about 20°C. and hatching commenced in the normal manner after about 5 or 6 days. Every second day after hatching commenced the newly hatched larvae were removed from the beaker for the purpose of being watered into the different pots. This was done by pouring the contents of the beaker on to a 60-mesh sieve held over a basin. The larvae passed through (helped by being washed through with tap water) and the cysts were retained on the sieve. Those cysts were tapped into a basin by quickly inverting the sieve over the basin and tapping its edge on the bench. Finally the cysts were washed back into the original beaker by fresh potato root extract and then replaced in the incubator. The larvae were watered on to the surface of the soil in the selected pots and in order that they may penetrate more readily to the deeper parts the soil in the pots was prodded with a pencil.

It was found that the hatching of the larvae in the laboratory extended over a much longer period than was expected. Judging from the abundance of larvae in the beakers, hatching seemed to be at its maximum about two weeks after it had commenced but it continued for a protracted period and had not quite finished after five weeks. The operation of watering larvae into the different pots was therefore performed every second day for about five weeks. In the case of pots 125 to 128 (Series C) and pots 135 to 138 (Series D) the operation commenced on April 23rd when the plants were just above ground, and in the case of pots 129 to 132 Series C and pots 139 to 142 (Series D) the operation commenced on May 14th when the plants were about 4 inches high.

Unfortunately, owing to a deficiency of cysts, only one pot (143) could be put in Series E. The plant in this pot was watered during the first month of its growth with potato root extract.

Hatching Experiments in Laboratory—Procedure and Results.

It has already been stated that the object of the hatching experiments was to test the rate of hatching of eelworm eggs in leachings from unsterilized soil growing potatoes compared with the rate of hatching in leachings from soil sterilized at different times and growing potatoes. It has also been stated that cysts of a large uniform size taken from the roots of potatoes grown in 1933 were used for the experiment.

It was felt that one hundred cysts should be used for each different extract (soil leaching) in order to get reliable results. Accordingly, on account of the large number of cysts being used it was decided to restrict the experiment to the following tests, using for each test ten lots of ten cysts each.

A. Hatching in leaching from unsterilized soil growing potato.
B. „ „ „ soil sterilized in Sept. growing potato.
C. „ „ „ „ „ Dec. „ „
D. „ „ „ „ „ Apr. „ „

The soil leaching was obtained by collecting the drainage from a pot filled with the required soil and growing a potato plant.

Deep watch glasses were used as containers for the different lots of cysts, ten cysts being placed in each. During the duration of the experiment the watch glasses were kept in an incubator at 20°C. At the time of each counting the cysts were first transferred by means of a fine wire loop from the watch glass containing them to another watch glass (perfectly washed) containing fresh extract. The larvae in the watch glass from which the cysts had been removed were then counted directly under a binocular microscope. The intervals between countings varied with the rate of hatching. When hatching was at its peak counts had to be made every day.

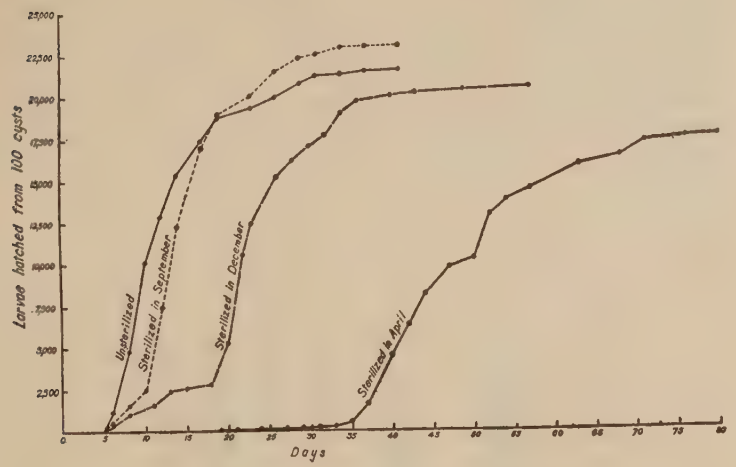
The hatching experiment was commenced early in April and counts were still being made up to the end of June.

Table IV.

Soil leaching.	Total hatch from 100 cysts after lapse of								
	5 days	10 days	20 days	30 days	40 days	50 days	60 days	70 days	80 days
A (unsterilized)	43	10,100	18,900	21,300	21,600				
B (sterilized Sept.)	43	2,500	20,000	22,500	22,600				
C („ Dec.)	0	1,400	5,300	17,200	20,100	20,500	20,600		
D („ Apr.)	0	0	15	85	4,600	10,300	15,300	17,200	17,800

It would serve no essential purpose to present such a lengthy table as would be required to show the rate of hatching day by day. The condensed table presented (with figures given to nearest hundred) serves the

purpose sufficiently well and the graph will help to visualise more clearly the rate of hatching in the different soil leachings.



The table and graph are so self explanatory that it is not necessary to pass comment on them.

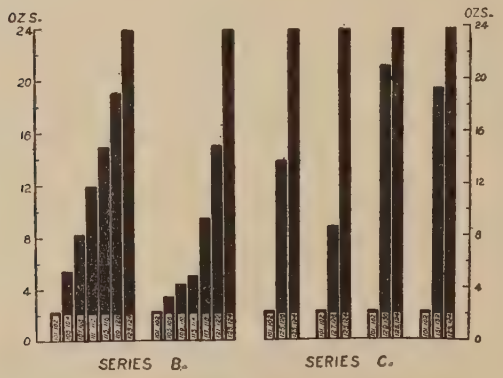
Pot Experiments—Results.

There is no need to present a lengthy dissertation on the appearance and condition of the plants in the different Series. The photographs on plate at end of this paper (taken during the first week of July) illustrate sufficiently well the extent to which the overground symptoms of potato sickness developed on the plants growing in soil containing eelworm cysts. The degree of root decay and the yield (ascertained during the last week of August) were in every case exactly in keeping with the overground appearance of the plant.

As was expected, cysts were abundant on the roots of plants growing in recently sterilized soil (and afterwards infected with cysts) although the plants showed little sign of potato sickness. Particular mention must be made of the pots into which newly hatched eelworm larvae were watered. In the case of all pots (both recently sterilized soil and unsterilized soil which had never grown potatoes) where watering in of larvae commenced

as soon as the sprouts appeared above ground symptoms of potato sickness developed quickly which were reflected afterwards in root condition, cyst abundance and yield. Where, however, the watering in of larvae did not commence until the plants were a few inches high little effect was produced on the growth and appearance of the plant but when the roots were examined at the end of August cysts were found in abundance.

As the yield of tubers was in every Series such a good indication of the intensity of potato sickness a special diagram showing the yields in Series B and Series C is presented.



In the case of pot 143 (Series E), where cysts were added to recently sterilized soil, the plant developed severe potato sickness which result was brought about by watering with potato root extract instead of ordinary water.

In the writers' opinion the fungus factor can be ruled out altogether in considering the results of the pot experiments because in all the pots containing soil which had been sterilized the amount of fungus infection (if any) was practically nil.

Conclusion.

The results of the pot experiments are clearly understandable in the light of the data obtained with the hatching experiments in the laboratory. The fact has been established that the root excretion of potatoes growing in recently sterilized soil does not possess the power of inducing the hatching of eelworm eggs in the normal manner. In other words, when eelworm cysts are added to sterilized soil the hatching of the eggs in these cysts does not commence until after the lapse of a much longer period than the normal, although potatoes are growing in the soil. The period elapsing before hatching commences is greatest in very recently sterilized soil (approximately 30 days in hatching experiment) and gradually becomes less according as the interval between sterilization and planting time is increased. The effect is practically non-existent in soil which has been sterilized six months.

The sterilization of the soil produces its effect on the potato root excretion and not on the eelworm eggs. This is obvious from pot 143 in Series E. In that case cysts were added to recently sterilized soil and experience had proved that if the plant growing in the pot was watered with ordinary water practically no symptoms of potato sickness would develop. Yet, severe potato sickness did develop as a result of the plant being watered with potato root excretion (leaching from unsterilized soil growing potato). Evidently the effect on the root excretion watered in was not operative on account of the large amount added at a time.

From the hatching experiments and the pot experiments it is obvious that, where hatching is delayed, the plants get a chance of making some growth and establishing a good root system before attack by the newly hatched larvae. The onset of eelworm attack only when a good root system has been established (see pots 129 to 132 Series C and 139 to 142 Series D) does not seem to have much effect on the further growth or productivity of the plants.

In view of what has been found with sterilized soil it appears reasonable to suggest that a somewhat similar condition of affairs may sometimes obtain in the field, where, due to one cause or another, plants make some growth and establish a good root system before the onset of eelworm attack. It can be seen from the experiments described in this paper that if hatching is delayed for even a short period after the plants commence to grow the obvious symptoms of potato sickness do not develop to any extent.

In the Rush district, Co. Dublin, early potatoes seldom suffer badly from potato sickness whereas second early and late varieties are often almost complete failures. The early varieties are planted as sprouted tubers very early in the year, evidently before the soil temperature is sufficiently high to permit of extensive hatching of eelworm eggs, and as a result the plants are large and well established before normal hatching commences. Many other details in connection with the field culture of potatoes may also result in the plants being well grown before they are attacked by eelworm larvae. Accordingly, in the light of the experiments described in this paper an explanation can be offered when one finds very healthy plants in the field with an abundance of eelworm cysts on the roots.

References.

Buckhurst, A. S. & Fryer, J. C. F., 1931.—" The problem of ' potato sickness ' : report upon certain experiments." *Ann. Appl. Biol.*, xviii, 584–601.

Carroll, J., 1933.—" A Study of the Potato Eelworm (*Heterodera schachtii*) in the Irish Free State." *Journ. Helm.*, xi, 137–156.

A.	Nos.	101 & 102,	Yield —	$2\frac{1}{4}$ ozs.
B.	,,	103 & 104,	,, —	$5\frac{1}{4}$ ozs.
C.	,,	107 & 108,	,, —	$7\frac{3}{4}$ ozs.
D.	,,	111 & 112,	,, —	$11\frac{3}{4}$ ozs.
E.	,,	115 & 116,	,, —	15 ozs.
F.	,,	119 & 120,	,, —	19 ozs.
G.	,,	123 & 124,	,, —	$23\frac{3}{4}$ ozs.

Only pots infected at the rate of 1 cyst per c.c. of soil are shown.

Journal of Helminthology, Vol. XIII, *No.* 2, *May*, 1935, *pp.* 91–102.

Observations on a Field Plot Experiment with *Anguillulina dipsaci* on Potatoes.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

In a previous paper the writer (1929) has given an account of the initiation and earlier work on a field plot experiment at Winches Farm devoted to the study of the stem eelworm, *Anguillulina dipsaci*, in its attacks on potato tubers. For the trials carried out in 1928 the original plot, which was 200 ft. long by about 21 ft. 9 ins. wide, was divided up into 30 smaller plots, each measuring 12 ft. long by 10 ft. 9 ins. wide, and 10 varieties of potatoes were tested, each variety being grown in triplicate. Following an inoculation of the soil with diseased tubers, it was found that all the varieties grown, whether earlies, second-earlies or main-crops, became infected. Since 1928 the plot has been cropped each year with potatoes and the purpose of the present paper is to put on record observations made and results obtained in subsequent years and to advance certain conclusions arrived at as a result of this work.

1929 Trials.

As the percentage infestation of the tubers lifted in 1928 was comparatively low, in some cases being in the region of 1% and in no case higher than 12·9%, it was decided to try and increase the chance of infection by adding more diseased material to the soil. For this purpose 2 tons, 6 cwt. of tubers attacked by *A. dipsaci* were obtained through the agency of a potato merchant in Lincolnshire. These were put through a mangold pulper and the pulp, after being evenly spread over the surface of the entire plot, was dug in early in April 1929. The same varieties as grown in 1928 were planted in triplicate and on the same small plots. Each small plot was set with 4 rows of potatoes, the rows being 2 ft. 6 ins. apart. Planting was carried out towards the end of April.

The crop was dug in October and stored in bags in a cool, dry but frost-proof place until February 1930 when the tubers were examined for the presence of disease and the accompanying parasite. The results are set

1929 Results.

Variety	Plot No.	Total No. of tubers	No. infected *A. dipsaci*	Percentage infection
Epicure	6	207	77	37·2
	9	219	71	32·0
	30	170	53	31·2
Sharpe's Express	4	251	92	36·6
	16	255	43	17·0
	27	277	93	33·57
Duke of York	3	186	71	38·1
	14	212	46	21·2
	21	182	46	25·2
Great Scot	2	242	56	23·14
	12	237	98	41·3
	29	206	90	42·7
British Queen	5	213	71	33·3
	13	351	96	27·2
	22	167	62	37·0
King Edward	10	262	77	29·3
	15	326	98	30·0
	24	257	67	26·0
Majestic	8	226	100	44·0
	19	259	136	52·5
	26	167	55	33·0
Arran Chief	11	301	127	42·3
	20	216	40	18·4
	23	212	70	33·0
Kerr's Pink	1	300	102	34·0
	18	321	82	25·8
	25	322	100	31·0
Up-to-Date	7	288	98	34·0
	17	297	114	38·3
	28	225	57	25·3

out in the first table where it can be seen that as a result of the application of the heavy dressing of diseased potato pulp a great increase in the incidence of disease was effected ; in some cases between 40%–50% of the tubers on a plot were infected. The diseased tubers were pulped and returned to the plot but owing to a misunderstanding the bulk of this material was dug into plots 1–4 instead of being spread over the whole area.

1930 Trials.

Within a day or two of planting the tubers the plot was given a uniform dressing of mixed mineral manures made up at the following rates per acre :—superphosphate of lime, 5 cwt. ; sulphate of ammonia, 2 cwt. ; sulphate of potash, 2 cwt. This was distributed by hand and was afterwards lightly hoed in. Additional quantities of sulphate of potash were applied to each two of the three replicate plots throughout so that one

1930 Results.

Variety	Plot No.	Total of tubers	Tubers with *A. dipsaci*	Percentage infected	Potash dressing
Epicure	6	236	27	11·4	4
	9	200	40	20·0	2
	30	253	30	11·8	1
Sharpe's Express	4	275	104	37·8	4
	16	252	18	7·1	2
	27	219	21	9·5	1
Duke of York	3	134	22	16·4	4
	14	310	48	15·5	2
	21	222	23	10·36	1
Great Scot	2	380	75	19·7	4
	12	425	63	14·8	2
	29	453	45	9·9	1
British Queen	5	381	111	29·1	4
	13	399	109	27·3	2
	22	518	66	10·8	1
King Edward	10	402	22	5·5	4
	15	410	35	8·5	2
	24	506	8	1·6	1
Majestic	8	359	126	35·0	4
	19	375	112	29·8	2
	26	354	33	9·3	1
Arran Chief	11	329	41	12·4	4
	20	417	43	10·3	2
	23	466	36	7·7	1
Kerr's Pink	1	242	41	17·3	4
	18	376	70	18·6	2
	25	403	38	9·4	1
Up-to-Date	7	349	38	10·8	4
	17	429	35	8·1	2
	28	386	21	5·4	1

received this artificial at the rate of 4 cwt. per acre and the other at 8 cwt. per acre. Carefully weighed quantities of the powdered substance were scattered evenly over the surface of the particular plot and were afterwards hoed in. By the use of these dressings it was hoped to test whether sulphate of potash, which has been claimed for many years to render red clover resistant to the attacks of *A. dipsaci*, would check or in any way affect the degree of infestation of potato tubers with the parasite.

The same varieties of potatoes were grown and the arrangement of the varieties according to plots was the same as in the two previous years. The crop was lifted in early autumn and the produce of each plot was separately bagged. The tubers were examined for the presence of disease during November 1930 and the results are set out in the accompanying table.

In general it can be seen that there has been a drop in the degree of infestation as compared with the previous year. The only exception to this is the case of British Queen on plot 13 where the percentage infection of 27·3 is practically the same as in 1929. When the figures for percentage infection are considered in relation to the sulphate of potash dressings, which are shown in the final column of the table, it is evident that the presence of double and quadruple dressings of this substance has in no way rendered the tubers less susceptible to attack since in most cases the plots receiving the double and quadruple quantities show a higher incidence of disease due to *A. dipsaci* than the plots receiving the single dressing. The diseased tubers were not returned to the plot since it was desired from now onwards to test the persistence of the infection in the soil. In this way it would be brought into line with ordinary farm practice where the crop is removed from the soil at the end of each season.

1931 Trials.

The plots were planted on exactly the same lines as in the three preceding years except that this year the following main-crop varieties were used :—King George, Flourball, Tinwald Perfection, White City, Abundance, Arran Consul, The Bishop, Ally, Ben Cruachan and Golden Wonder. Planting was carried out early in May and each variety was planted in triplicate as in the earlier trials. The crop was lifted in the autumn and stored in bags through the winter. The examination of the tubers for

the presence of disease was carried out early in 1932. Instead of the entire produce of each plot being counted and examined as in previous years, 100 tubers were taken from each bag by random sampling, *i.e.*, without conscious selection as to size and appearance. The results are set out in the accompanying table.

1931 Results.

Variety	Plot No.	Tubers examined	Tubers with *A. dipsaci*	Percentage infected
Tinwald Perfection	4	100	0	0
	16	,,	1	1
	27	,,	0	0
White City	7	,,	3	3
	17	,,	1	1
	28	,,	2	2
The Bishop	11	,,	0	0
	20	,,	1	1
	23	,,	0	0
Ally	3	,,	6	6
	14	,,	6	6
	21	,,	2	2
Abundance	1	,,	0	0
	18	,,	5	5
	25	,,	1	1
King George	2	,,	9	9
	12	,,	1	1
	29	,,	1	1
Flourball	6	,,	2	2
	9	,,	1	1
	30	,,	1	1
Golden Wonder	10	,,	2	2
	15	,,	2	2
	24	,,	0	0
Arran Consul	8	,,	2	2
	19	,,	1	1
	26	,,	0	0
Ben Cruachan	5	,,	0	0
	13	,,	2	2
	22	,,	1	1

The degree of infestation is, on the whole, very light and shows a marked decrease compared with the previous year. All the new varieties tested

were found to be susceptible to attack and this fact, taken in conjunction with the results obtained in the earlier trials, would seem to warrant the conclusion that most of the potato varieties in commercial cultivation are susceptible to attack from the parasite.

1932 Trials.

The original experimental field plots were found to be too large for convenient working and were all reduced to about half their size by making them a little more than 100 ft. long instead of 200 ft. In consequence of this instead of the 30 plots there were now only 16 under experimental cultivation. Each small plot was planted with the same variety it had had in 1931, the seed having been saved from the 1931 crop. Planting

1932 Results.

Variety	Plot No.	Tubers examined	Tubers with *A. dipsaci*	Percentage infected
Tinwald Perfection	4	100	1	1
	16	,,	3	3
White City	7	,,	2	2
The Bishop	11	,,	1	1
Ally	3	,,	3	3
	14	,,	3	3
Abundance	1	,,	10	10
King George	2	,,	6	6
	12	,,	2	2
Flourball	6	,,	0	0
	9	,,	1	1
Golden Wonder	10	,,	11	11
	15	,,	9	9
Arran Consul	8	,,	3	3
Ben Cruachan	5	,,	1	1
	13	,,	0	0

was carried out about the middle of May and the plots were dug in the autumn. As in previous years, the produce from each plot was bagged up separately and examined later for the determination of the percentage infection. The results are set out in the above table.

It will be noticed that the degree of infection is still quite light but is, on the whole, somewhat higher than in 1931. It is difficult to suggest an adequate reason for this. Possibly it is due to the residuum of infective material remaining in the soil or to its re-introduction by means of the tubers planted carrying a light infestation which escaped notice. This latter suggestion is considered to be rather improbable as the tubers had been carefully examined prior to selection for setts and were quite healthy in appearance when sprouted just before being planted.

1933 Trials.

As all the varieties of potatoes tested appeared to be susceptible to attack from *A. dipsaci*, it was decided to vary the crops grown on the small plots during this season. Only 8 of them were planted with potatoes, the

15	Barley	16	Potatoes Tinwald Perfection
13	Potatoes Ben Cruachan	14	Onions
11	Oats	12	Potatoes King George
9	Potatoes Flourball	10	Rye
7	Turnips	8	Potatoes Arran Consul
5	Potatoes Ben Cruachan	6	Peas
3	Mangolds	4	Potatoes Tinwald Perfection
1	Potatoes Abundance	2	Wheat

remaining 8, alternately arranged amongst them, as shown on the accompanying plan, being sown with the following crops :—wheat, rye, barley, oats, mangolds, peas, turnips and onions, all of which have been recorded as hosts of *A. dipsaci*. It was thought that it would be useful to test whether any of them would prove susceptible to the strain of the parasite attacking potatoes, though it must be admitted, in view of the lightness of the infestation on potatoes on most of the plots, that it was hardly

expected the various other crops would exhibit any marked symptoms of attack. Nevertheless since some of these crops might be used in a rotation with potatoes it was considered useful to test them.

The 8 small plots were planted with potatoes early in May, each with the same variety it had borne in the previous year and with setts saved from the 1932 crop. The other crops were also sown early in the same month but all the cereals made a poor show owing to the ravages of rooks. Some few plants, however, on each cereal plot managed to grow. The other crops, mangolds, peas, turnips and onions grew quite well. Examination of these and of the cereals at various times throughout the summer and early autumn failed to reveal any sign of discoloured, swollen or stunted stems such as are normally associated with attacks of *A. dipsaci* and it was concluded that they had remained unaffected by the parasite.

The potato plots were dug in the autumn and the tubers bagged up separately for later examination which was carried out as in the two previous years by the examination of 100 tubers taken at random. The results are set out in the table below.

Variety	Plot No.	Tubers examined	Tubers with *A. dipsaci*	Percentage infected
Abundance	1	100	25	25
Tinwald Perfection	4	,,	5	5
Ben Cruachan	5	,,	3	3
Arran Consul	8	,,	0	0
Flourball	9	,,	1	1
King George	12	,,	1	1
Ben Cruachan	13	,,	2	2
Tinwald Perfection	16	,,	2	2

The degree of infection is quite low except in the case of plot 1 where 25% of the tubers showed the presence of the parasite as compared with only 10% in 1932 and 0 in 1931. The writer is unable to advance any satisfactory explanation for this beyond the suggestions put forward earlier in commenting on the increase to 10% shown by this plot in the 1931 trials.

1934 Trials.

The plots were planted as in 1933; 8 with potatoes and 8 with other crops and the plan drawn up for that season was followed in this also. The potatoes were planted at the end of April and the seeds of the other crops were sown at about the same time. Birds again played havoc with the cereals, in spite of the plots being netted, with the result that there were very poor stands of wheat and oats; the rye and barley were not so badly attacked. Examination throughout the growing season of the mangolds, turnips, peas and onions as well as the cereals failed to reveal any sign of disease caused by *A. dipsaci*. The potatoes were lifted at the end of August which is rather earlier than the usual time of lifting but was due to the very dry summer which had caused the early ripening of the tubers. The produce from the 8 potato plots were bagged up separately and were examined for the presence of disease at the end of October. Owing to the low percentage infection shown by the tubers in the previous season, it was decided to examine all the tubers from each plot instead of a random sample of 100. The results are set out below.

Variety	Plot No.	Tubers examined	Tubers infected	Percentage infection
Abundance	1	345	40	11·6
Tinwald Perfection	4	314	7	2·26
Ben Cruachan	5	293	0	0
Arran Consul	8	238	0	0
Flourball	9	148	0	0
King George	12	218	0	0
Ben Cruachan	13	258	0	0
Tinwald Perfection	16	239	·2	0·83

It will be seen that in five out of the 8 plots none of the tubers were infected. In plot no 16 the infection is below 1% whilst in plots Nos. 1 and 4, which in the previous year had infections of 25% and 15% respectively, the infection has fallen to 11·6% and 2·26%. It will be noticed

that plot no. 1 shows again the highest degree of infection and again it is impossible to suggest a satisfactory explanation. It seems scarcely reasonable to suggest that the tubers planted were infected since one would expect the same factor or error to have been operative in the case of the other plots. On the whole it seems more feasible to suggest that for some reason or other, at present not understood, plot no. 1 has carried a heavier residual infection than any other plot. This, however, can scarcely have been due to tubers accidentally left in the soil at the time of lifting the crop since as much care was exercised in picking up the tubers on this plot as on the others.

Discussion and Conclusions.

Following the application of the dressing of pulped diseased tubers early in 1929 there was a heavy infestation in the tubers of the 1929 crop over all the plots. In 1930 the attack was lighter than in 1929. The incidence of disease was rather irregular ; in some cases the decrease in percentage infection is considerable, in other cases only slight. In 1931 the degree of infestation was low throughout, the highest percentage infection being 9 and in several cases there was no infestation detectable. From 1932 onwards, plots 17 to 30 inclusive were taken out of experimental cultivation but if we consider the results from the 8 plots carrying potatoes, apart from plot 1, in particular, which presents anomalous results difficult to account for, it can be seen that there has been a further gradual loss of infection. Plots 10 and 15 also present results of a somewhat similar character to plot 1, at any rate in 1931 and 1932, for in each case there was an increase in percentage infection from 2 to about 10.

Bearing these exceptional results in mind, it seems fairly safe to conclude that even where potatoes are grown on the same land year after year the degree of infestation with *A. dipsaci* declines rather than increases. This is doubtless because with the lifting of the crop the main source of infection is removed at the same time. Whether the same would hold good for commercial cultivation is perhaps doubtful for there the ridges are split by ploughing and the tubers are hand picked. There would therefore be greater risk of tubers being overlooked and left in the soil than in experimental plots which are carefully dug by the fork. Such tubers as are left in the soil would doubtless constitute a residual source of infection.

The anomalous results, however, presented by the figures for percentage infection of tubers on plot no. 1 in the seasons 1932, 33 and 34 suggest that the true understanding of the nature of residual infection in the soil has yet to be determined.

Plot	Percentage Infections					
	1929	1930	1931	1932	1933	1934
1	34·0	17·3	0	10	25	11·6
2	23·14	19·7	9	6	wheat	wheat
3	38·11	16·4	6	0	mangolds	mangolds
4	36·6	37·8	0	1	1	2·26
5	33·3	29·1	0	1	3	0
6	37·2	11·4	2	0	peas	peas
7	34·0	10·8	3	2	turnips	turnips
8	44·0	35·0	2	3	0	0
9	32·0	20·0	1	1	1	0
10	29·3	5·5	2	11	rye	rye
11	42·3	12·4	0	1	barley	barley
12	41·3	14·8	1	2	1	0
13	27·2	27·3	2	0	2	0
14	21·2	15·5	6	3	onions	onions
15	30·0	8·5	2	9	oats	oats
16	17·0	7·1	1	3	2	0·83

The application of double and quadruple dressings of sulphate of potash to the plots in 1930 apparently afforded no protection for the tubers against attack by the parasite.

Although grown in soil admittedly lightly infested with the parasite, it has been found that the four cereals, wheat, barley, rye and oats and also mangolds, turnips, peas and onions are not attacked by the strain of the parasite attacking potatoes. It would be unwise to draw any conclusion as to the susceptibility of these crops to the potato strain of *A. dipsaci* until further experiments have been carried out under conditions ensuring an abundant supply of infective material. At the same time one may tentatively suggest that under a system of four course or longer rotation there would be little risk of any of the cereal or root crops becoming attacked by the potato strain of *A. dipsaci* and the same would probably hold for most of the leguminous crops such as clovers, peas, beans and lucerne.

If, as these trials seem to indicate, there is a decreasing risk of attack from the parasite even when potatoes are grown on the same land year

after year, it is asked how the parasite maintains itself and is spread from place to place, the answer is that it is probably by means of lightly infected tubers which escape detection and are distributed as " seed " potatoes.

Reference.

Goodey, T. 1929.—The Stem Eelworm, *Tylenchus dipsaci* (Kühn, 1858) ; Observations on its attacks on Potatoes and Mangolds with a Host-list of plants parasitized by it. *J. Helminth.*, vii (4), 183–200. (*W.L.* 11224b.)

Journal of Helminthology, Vol. XIII, *No.* 2, *May*, 1935, *pp.* 103–114.

The Second Ecdysis of the Infective Larvae of Certain Trichostrongylidae in Solutions of Sodium Sulphide and of Organic Compounds containing Sulphur.

By Geoffrey Lapage, M.D., M.Sc.
(*Institute of Animal Pathology, University of Cambridge.*)

I. Introduction.

In the course of the writer's (1935) work on the second ecdysis of the infective larvae of nematodes parasitic in sheep some effects of solutions containing sulphides upon these larvae were observed. These seem to merit further investigation. The effects of these solutions upon the permeability of the sheaths of infective larvae seem especially likely to provide valuable information. These sheaths are the cast-off and presumably dead skins of the second larvae, so that any information we gain about them may apply also to the skins of the other larvae, whether these are parasitic or not, and perhaps to the peculiar cuticle of the mature worms. It may therefore help us towards methods of rendering both the larvae and the worms more permeable to anthelmintics used against these worms. In any event, it will help us to a better physiological knowledge of these serious pests of animal and human life.

II. Material and Methods.

The larvae used for this work were those of *Haemonchus contortus*, *Ostertagia circumcincta*, and species of *Trichostrongylus*. They were reared by the method described by the writer (1933a) upon pure cultures of a strain of B. coli recovered from the intestines of first parasitic larvae of these species, excepting a few which were reared in the usual way upon partially sterilised faeces.

The larvae were studied in hanging drops of the kind used for tissue culture, one advantage of this method being that the drops can be sterilised if necessary. Some were studied in " solid " or ordinary watch glasses.

The solutions used were watery extracts of garlic, solutions of sodium sulphide, taurocholate of soda, cystein hydrochloride, allyl sulphide, sulphonal, saccharin and sodium thiosulphate. It should be noted here, in view of the fact that B. coli and other bacteria may produce H_2S, that some B. coli were probably introduced into all the preparations now to be described, from the cultures in which the larvae had been reared, whether these were washed before they were put into the media or not. The strain of B. coli used was the one described as No. 951 in the writer's earlier paper (1933a). Most of the B. coli in the cultures may have been dead at the time the larvae were removed for these experiments, because the larvae used had been mature in the cultures for about a month and the cultures had been inoculated with the B. coli 47 days before these experiments began. In some of the drops, however, the bacteria visibly multiplied, even at room temperature, so that their effect on the media must be considered.

III. Infusions of Garlic.

The first of these solutions used was a watery extract of garlic, made by soaking 1 gramme of chopped up garlic bulbs in 20 cc. of tap water at room temperature. The treatment of larvae with this was suggested by the statements by Mudie (1934) and Johnston (1934) that the addition of garlic to human diet will cause a disappearance of threadworms from the alimentary tract. It was thought worth while to see whether the effect of garlic upon nematode larvae would give any evidence in favour of this rather sweeping statement. At room temperature in an infusion used the day after it was made no effect on the larvae was seen and they died in it in 2–3 days. When, however, the same infusion was used 4 days after it had been made and kept at room temperature and the sealed hanging drops were incubated at 38°C., 25% of the larvae had exsheathed the morning after they were put into it, 70% exsheathed in 2 days, and only a few did not exsheath at all. The pH of the infusion 6 days after it was made was 6·8–7. It had not been sterilised in any way. Most of the exsheathed larvae were dead by the 5th day, but some were still active on the 8th.

In another series of drops made under the same conditions none of the larvae had exsheathed the day after they were put into the infusion, but 66% exsheathed on the second day and all had exsheathed on the third. All but two of the exsheathed larvae were dead on the 9th day.

A remarkable feature of all these observations was that the empty sheaths all disappeared very quickly and some of the exsheathed larvae also quickly disintegrated and disappeared soon after death, but were unaffected before it. No particular change was observed in the sheaths before the larvae exsheathed, so that it was difficult to ascribe these ecdyses to the effects of any alkali that might be present in the infusion, especially as it was slightly acid in reaction, although the salts present might have contributed an osmotic effect. It seemed more important to test the effect of oil of garlic and some allyl sulphide was therefore obtained from British Drug Houses, Ltd.

IV. Effects of Allyl Sulphide.

The effects of this have not yet been fully tested. Such experiments as have been made have not been satisfactory, because the oil is not readily miscible with water. 0·5 cc. of the oil was, however, shaken up with 9·5 cc. of distilled water and larvae were put into the mixture, which smelt strongly of garlic. At room temperature only 1 larva out of about a dozen exsheathed between the 8th and the 9th day, although the larvae all remained active for 18 days. Practically no effect was observed upon the sheaths or the larvae, except a certain amount of retraction of the larvae, which was not considered abnormal.

At 38°C., in one series, only one larva exsheathed, and it required practically 24 hours to get out of its sheath, probably because it was only feebly active, as were all the others; and all the larvae were dead in 4–5 days.

In another series at 38°C., two larvae half-emerged from their sheaths on the second day, and another between the fourth and fifth days, but all these were too feeble to emerge completely and were practically dead when the caps were detached. Most of the other larvae were also dead in 5 days. At this temperature, however, it was observed that many of the sheaths were thinner than normal, whether the larvae exsheathed or not, and the impression was gained that, if the larvae had been more active, they might have broken their way out. It was felt, however, that not enough allyl sulphide was present to exert any constant effect, and meanwhile collateral observations upon the effect of sodium sulphide and organic compounds containing sulphur were claiming time and attention.

V. Effects of Sodium Sulphide.

In a 1% solution of this in distilled water a remarkable effect was seen even at room temperature. All the sheaths of all the larvae were almost immediately distended and looked extremely thin, their appearance resembling that of larvae in 1% and 2% NaOH (Lapage (1935)), except that it was more marked. There could be no doubt whatever that these sheaths were distended, because the larvae looked small within what were now diaphanous tubes to the ends of which neither their heads nor their tails could reach. In one series of preparations some of them exsheathed in about 25 minutes and 40% had exsheathed the next morning, the others being active in their swollen thin sheaths. No further ecdyses, however, occurred, although some larvae were still feebly active in their sheaths after 33 days at room temperature. Most had, however, died by the 16th day. The addition of 0·5% NaOH did not cause more ecdyses, nor did the addition of 0·5 HCl. It appeared that the effect of this solution was not further exerted after the first 24 hours.

In another series of similar preparations some larvae also exsheathed within the first 45 minutes, and the same immediate distension and thinning of the sheaths was observed in all the larvae. The morning after they had been put in, 12% had exsheathed. The preparations were then incubated at 40°C., but only 4 more exsheathed, two of them 7 hours after the rise of the temperature, and two 24 hours after the change. No more exsheathed till the 9th day, when one exsheathed. Two were exsheathed on the 12th day and by the 16th day both sheathed and exsheathed larvae were all dead. Here again the main effect of the solution was exerted at once, little further effect being seen after the second day.

This happened also in a further series, kept throughout at 39°C. In these drops all the larvae except one exsheathed within 25 minutes and the same effect upon the sheaths was seen. Most of the exsheathed larvae were active for 7 days in the solution and then died off by one by one, until on the 16th day only one was left active, when the one which had not exsheathed was also dead in its thin and swollen sheath.

The conclusion was therefore drawn that this solution exerts its effect at once, or within the first 2 days, and the reason for this is perhaps that it is an effect due to the volatile H_2S in it, of which it smells strongly. If this is true, it is interesting because H_2S is among the decomposition

products produced by bacteria in the intestine, especially in certain states of disorder of this organ. Little evidence has so far been obtained as to how it acts on the sheaths, but the morphological effects of its action resemble those of chlorine described by the writer (1935). It is possible that H_2S alters, as chlorine does, the permeability of the sheaths, so that the internal pressure is increased and the sheath distended. Its action differs from that of chlorine, however, in two important respects. It does not stimulate the larvae to increased activity so that the ecdyses are helped, but renders them slowly less active so that if they do not exsheath at once, they cannot apparently do so, and it does not apparently render the sheaths soluble in NaOH as chlorine does. There was probably enough NaOH present in the solutions used to be responsible for the thinning of the sheaths seen, but the following considerations suggest that the thinning was not due to a solvent action of NaOH but chiefly to the stretching of the greatly distended sheath.

The sheaths in this series of preparations were not dissolved, even after 33 days at 39°C., although they were often so thin and diaphanous that they could only be seen with difficulty, and sometimes higher magnifications were necessary to distinguish between sheathed and exsheathed larvae.

The sheaths were not dissolved when NaOH was added after distension had occurred, nor were they dissolved in a stronger solution of Na_2S, made not with water but with a 0·5% solution of NaOH, into which about 10% of Na_2S was put. This resulted in an instant and enormous distension of the sheaths, without, however, causing any ecdyses, probably because the larvae became immediately very feebly active and were all dead in 24 hours. This demonstrates not only the great tensile strength of the sheaths, but also the necessity of some activity of the larvae for successful ecdyses. These larvae, however, were studied only at room temperature.

It seems likely therefore that, if this effect is due to H_2S, the change in the sheath produced is different from that produced by chlorine and that therefore a different solvent is required after it to dissolve the sheaths. Whether the effect of the garlic infusion is also exerted in this way has not yet been determined. One reason why more ecdyses occur in these infusions than in sodium sulphide, is probably that their higher salt content contributes osmotic effects which facilitate the ecdyses.

VI. Effects of Cystein Hydrochloride.

The effects of watery solutions of this act differently than those of sodium sulphide in one respect, namely, that, although they cause some blistering or localised swelling of the sheaths, they do not cause, as a rule, the marked general distension seen in sodium sulphide solutions. The reason for this, however, may have been simply that the solutions used were so much weaker, 0·1% solutions of cystein hydrochloride being used, while a 1% solution of sodium sulphide was the weakest used.

Cystein hydrochloride was tried because the sulphur-containing amino-acid cystine occurs in the alimentary canal, and because bacteria which produce H_2S do so more actively if cystine is added to their enviroment. It was hoped, therefore, that, even if cystein were added and did not itself affect the sheaths, effects similar to those produced by sodium sulphide solutions might be produced if B. coli were added to the medium, either from the culture in which the larvae had been reared, or from a separate culture of it. Cystein, with and without various amino-acids, had been previously tried in attempts to cultivate further the parasitic larvae, but without success.

The 0·1% solution which had been used in attempts to cultivate the parasitic larvae was first tried. This had been kept sterile at room temperature for 15 months. The larvae were not washed before they were put into it, so that some B. coli were introduced with them, and to the naked eye they appeared to multiply, even at room temperature.

At room temperature, however, no larvae had exsheathed after 18 days, when most of the larvae were only very feebly active. The sheaths were not thinned, nor distended, but had, even 2 hours after they were put into the solution, a curious furry appearance, their clearly defined outlines having been lost. Fine particles readily adhered to them, so that they appeared encrusted with bacteria, and they showed a tendency to collect in clumps, several of them writhing in a tangled group from which they did not extricate themselves, even when they died. This clumping and " stickiness " is sometimes also seen in very acid media, and Fülleborn (1932) has noted similar " agglutination " of the larvae in media containing serum. It requires further investigation.

At 38°C., however, all the larvae sooner or later exsheathed, throwing off the typical caps. The ecdyses were, in fact, preceded by changes

similar to those recorded by the writer (1935) in mixtures of NaOH and HCl, the sheath swelling at the base of the cap and showing a refractile ring where the cap was later detached. A few only half-emerged and died thus, and a few were so feebly active that they needed a day or so to get out of the sheaths; in some instances, indeed, the "stickiness" of the sheaths appeared to be the cause of this delay. The sheaths quickly acquired the furry appearance described above and blisters, that is to say, marked local distensions rather than the uniform swelling that appears in HCl and NaOH mixtures, appeared often in the region of the base of the cap, as they do in chlorine water. The sheaths had, however, the transparency that they acquire in strong solutions of NaOH and in solutions of sodium sulphide. The anterior ends of sheaths from which the cap had been detached were often much more clearly defined than usual, as they may be also in sodium sulphide solutions. They were, however, often not dissolved even after 21 days at 38°C. in this solution, but in some preparations at 38°C. they were so markedly thinned that the appearance at any rate of partial dissolution was given and the larvae appeared to be trying to break out of them; and in a freshly made similar solution many were dissolved (see below). The effect of this solution, as judged by the number of ecdyses in it each day, was apparently not exerted chiefly on the first two days, as that of the sodium sulphide appeared to be, but went on progressively, so that almost each day some larvae exsheathed, or began to show appearances like those seen in mixtures of NaOH and HCl preparatory to exsheathing, until at the end of 6 or 7 days at 38°C. all of them had exsheathed. The exsheathed larvae lived well in this solution, most being healthy in it for 6–10 days.

Another series were put into a freshly made 0·1% solution of cystein hydrochloride in the 1 in 10 dilution of plain broth which was being used for the cultivation of the larvae on B. coli. This was inoculated with the strain 951 of B. coli on which the larvae had been reared, and was incubated overnight, when the larvae were put into it, direct from the cultures of them, without previous washing. These preparations were kept at 38°C. throughout and the B. coli were plentiful in them. Much the same effects were observed, but the number of ecdyses obtained was less than in the preceding series, some of the larvae dying without exsheathing. Most of the sheaths were, moreover, dissolved completely, a difference of effect which is puzzling.

VII. Effects of Sodium Taurocholate.

Solutions of this had been tried earlier, both alone and in combination with artificial pancreatic juice. It had also been tried during the attempts to cultivate further the parasitic larvae. As in the other preparations, some B. coli were present in all the drops of it used.

The old 1% solution previously used was also first tried and at room temperature no ecdyses occurred in it and no effect on the larvae could be seen. At 38°C., moreover, it did not cause swelling of the sheaths, nor did it blister them. It did not immediately thin them either, but the sheaths of some larvae looked thinner after 5–6 hours. The empty sheaths after ecdyses were not dissolved by this solution. Some ecdyses occurred, but more were only half completed, the larvae failing to emerge after the caps had been detached. Changes in the sheaths similar to those which occur in mixtures of NaOH and HCl were seen, but often the caps remained attached to the sheaths, or even remained, when they were completely detached, on the heads of the larvae, which were too sluggish to withdraw from them. There was no visible evidence of high internal pressure, like that seen in hypochlorite solutions, and the larvae were never propelled halfway out of their sheaths, as they are, for example, in diluted Milton. The exsheathed larvae, moreover, lived only 5–6 days in this solution and those that failed to exsheath were mostly dead in the same time. It was therefore concluded that this solution was more lethal than the cystein or sodium sulphide, a conclusion which agreed with that drawn from earlier experiments with both infective and parasitic larvae, to which taurocholate seemed to have been toxic.

Another series were then studied in a freshly made 1% solution of sodium taurocholate, and in this the effects were similar. One or two larvae exsheathed in it, however, at room temperature, but required 12 days to do so. At 38°C. the same failure to exsheath was seen, although some caps were detached, and many showed the swelling and refractile ring and other changes seen in mixtures of NaOH and HCl. The only apparent reason for the failure of the larvae to exsheath was their sluggishness, so that this solution also suggested that taurocholate is in some way toxic to the larvae.

The effects of three other solutions may be briefly noted also, although any interpretation of these results must await the results of the other experiments now being done with other sulphur-containing compounds.

VIII. Effects of Saccharin.

In a 1% solution of this in water, in which plenty of B. coli were present, 75–100% of the larvae exsheathed in 7–8 days. Usually 33–45% had exsheathed on the first or second days, after which an interval without any ecdyses occurred till about the 6th to the 9th day, and then very few more occurred. This suggests that, if the effects were due to H_2S, the initial concentration of this was followed in about 4 days by the slow accumulation of more, which may have been produced by the bacteria. The larvae that failed to exsheath lived on for a total of 18 days or so in the solution. The exsheathed larvae lived only a few days in it. The ecdyses were preceded by swelling of the sheaths at the oesophageal region and by similar changes to those seen in HCl and NaOH, but the general distension seen in solutions of Na_2S was not observed. The empty sheaths were not so markedly thinned, nor were they visibly changed after 20 days or more at 38°C. The general effects of this solution were, therefore, like those of cystein hydrochloride and it did not appear to be particularly toxic to the exsheathed larvae, although these did not live so well in this as in the sodium sulphide solutions.

IX. Effects of 0·2% Sulphonal.

The solubility of this in water prevented the use of a stronger watery solution than this. The weak effects of it are possibly, like those of allyl sulphide recorded above, due to this.

At room temperature no visible change occurred other than considerable retraction of the larvae in their sheaths ; and none of the larvae exsheathed. The retraction of the larvae was not measured, but it was obviously not due to distension of the sheath, but to shrinkage of the larvae. It occurred also to some of the larvae at 38°C. It is difficult to understand why it should occur in so weak a solution, if it is due to osmosis alone.

At 38°C. some larvae showed thinned and distended sheaths, the thinning appearing in some in a few hours, and the distension being general, affecting the whole sheath, so that it resembled that seen in solutions of sodium sulphide, rather than the localised blistering seen in the other solutions in this series. About 50–75% of the larvae had exsheathed after 10 days. Most of them exsheathed about the 5th day and it is interesting to note that, although the refractile ring at the base of the cap was often

visible, caps were not always detached, but some empty sheaths showed the ragged edges of an opening at the anterior tip, like that seen when ecdyses occur in Milton. This, together with the generalised distension, and the fact that the larvae were not very much more active than they are in the other solutions described in this paper, suggests that an osmotic internal pressure operates in this solution as it does in hypochlorite solutions, and that in this respect the effects of this solution differ from those of sodium sulphide, cystein and taurocholate. Another fact pointing the same way is the observation that the empty sheaths were all dissolved slowly even in this weak solution of this compound, so that some alteration of their permeability must have occurred.

Some of the exsheathed larvae, moreover, lived as long as 16 days in this solution, so that it is even less toxic to them than sodium sulphide. Whether it would be so in stronger solutions, if these could be obtained in a medium not itself toxic to the larvae, remains to be seen.

X. Effects of Sodium Thiosulphate.

Some larvae were put into a 1% solution of this and, even at room temperature, marked shrinkage of the larvae inside their sheaths occurred, although the sheaths were not visibly altered at this temperature, and no ecdyses occurred.

At 38°C. retraction of the larvae also occurred, the outline of the larvae being often irregular and their heads and tails crumpled. Some of them occupied only about half of the interior of their sheaths even after 1 day in the solution. Most of them became sluggish and were inactive, or only very feebly so by the second day. In spite of this inactivity, however, some ecdyses were seen on the 4th day, but these were very few in comparison with those seen in the solutions just described. Many of them failed because the larvae were inactive and did not emerge when the cap was detached. The exsheathed larvae quickly died and the larvae that failed to exsheath were all dead in 6–7 days, some dying in 2 or 3 days.

The sheaths of many of those which failed to exsheath were thin and diaphanous and generally distended away from the larvae within, as they may be in sodium sulphide or NaOH solutions, except that in thiosulphate the larvae were crumpled also. Sometimes these distended

sheaths collapsed later, so that the larvae lay dead in a crumpled disphanous shroud, or, if they were still active, appeared to be trying to break their way out of them, an appearance seen also in NaOH and other solutions and described elsewhere (Lapage (1935)). In other instances localised distensions at the base of the cap were seen after 2 or 3 days, the appearances resembling those produced by the vapour of acetic-Milton (Lapage (1935)). Neither the empty sheaths, nor those on the larvae which failed to exsheath, were, however, completely dissolved, although they often became, after 7 or 8 days, so thin that they could only be detected with difficulty.

There was obviously, therefore, some alteration of the sheaths by this solution, and the distension may have been due to the alteration of the permeability caused by this; but it is difficult to understand why shrinkage of the larvae should also occur, unless this was due to a chemical action of this salt.

It would be premature to draw any conclusion from the effects of the solutions here described until the results of many more experiments with them and with other sulphur-containing compounds have been made. It does not yet seem clear that the effects above described are in reality due to the sulphur or its compounds in them; but the effects of sodium sulphide solutions, and those of cystein hydrochloride in which B. coli was present, do suggest that H_2S may have an effect on the sheath comparable in its influence on the osmotic factors controlling the ecdyses to that produced by chlorine and hypochlorites, although it may be different chemically, so that the sheaths altered by it are not, for example, thereby rendered soluble in alkalis. Whether most of the effects seen in the other solutions here described are due to NaOH present in them, and not to their sulphur compounds, needs further investigation.

Sodium sulphide solutions and probably H_2S water also could certainly be used as a means of artificially producing ecdyses, and the parasitic larvae would live for a time in them after exsheathing; but the 1 in 20 dilution of Milton used by the writer (1933 b) for this purpose is better because it also sterilises the parasitic larvae which it sets free. It is also more easily prepared and pleasanter to use.

The possibility that, in view of these effects, solutions containing sulphides might be used to kill either the infective larvae or the parasitic larvae, or the " adult " parasites, awaits investigation.

SUMMARY.

1. The second ecdysis of infective nematode larvae readily occurs in watery infusions of chopped up garlic bulbs and the cast sheaths are dissolved after the larvae have left them. No particular change in the sheaths was observed before the ecdyses occurred.

2. Ecdyses also occurred readily in 1% sodium sulphide in distilled water, the sheaths being, however, remarkably distended and thinned before the ecdysis occurred.

3. In 0·1% solutions of cystein hydrochloride ecdyses occurred with changes in the sheath similar to those recorded by the writer in mixtures of HCl and NaOH and leading to detachment of a cap, but with little distension or thinning of the sheaths. The cast sheaths were usually not dissolved. Similar ecdyses occurred in 1% sodium taurocholate and 1% saccharin in distilled water, but the former of these two solutions seemed more toxic to the larvae than either saccharin or cystein hydrochloride.

4. Some ecdyses occurred in 0·2% sulphonal with detachment of a cap and some osmotic distension and thinning of the sheaths. The empty sheaths were all slowly dissolved in the medium.

5. In 1% sodium thiosulphate a few ecdyses occurred with some thinning and distension of the sheaths, but the larvae were usually shrunken and often died quickly.

6. The solutions here described could be used for artificial production of ecdyses, 1% sodium sulphide being probably the best for this purpose ; but none are so useful as the 1 in 20 dilution of Milton hypochlorite used by the writer (1933 b), because this also sterilises the exsheathed larvae and the medium in which they exsheath.

REFERENCES.

FÜLLEBORN, F., 1932.—" Ueber die Taxen und der infektionsfähigen Larven von Strongyloides und Ancylostoma." II. Mitteilung. *Zbl. Bakt.*, 126, 161–180. (*W.L.* 23684.)

JOHNSTON, F., 1934.—" Threadworms." *Brit. med. J.*, No. 3813, 224. (*W.L.* 3579.)

LAPAGE, G., 1933a.—" The Cultivation of Infective Nematode Larvae on Pure Cultures of B. coli." *3rd Rep. Dir. Inst. of Animal Path., Univ. of Cambridge*, 237–270.

——, 1933b.—" Cultivation of Parasitic Nematodes." *Nature*, 131, 583–4. (*W.L.* 14900.)

——, 1935.—" The Second Ecdysis of Infective Nematode Larvae." *Parasitology*. 27, 186–206. (*W.L.* 16035.)

MUDIE, E. C., 1934.—" Threadworms." *Brit. med. J.*, No. 3813, 224. (*W.L.* 3579.)

Journal of Helminthology, Vol. XIII, *No.* 2, *May*, 1935, *pp.* 115–128.

The Behaviour of sterilised exsheathed infective Trichostrongylid larvae in sterile media resembling their environment in Ovine hosts.

By Geoffrey Lapage, M.D., M.Sc.

(*Institute of Animal Pathology, University of Cambridge.*)

I. Introduction, Material and Methods.

From time to time since the writer (1933b) found that a 1 in 20 watery dilution of Milton will not only cause the second ecdysis of infective nematode larvae, but will also sterilise the first parasitic larva released by that ecdysis, these exsheathed parasitic larvae have been put into various sterile media, in an endeavour to find a medium in which they would develop further. The larvae used for these experiments have been those of *Haemonchus contortus*, *Ostertagia circumcincta* and species of *Trichostrongylus*, reared upon pure cultures of a strain of B coli recovered by the writer (1933a) from the intestines of the parasitic larvae themselves. The larvae were studied chiefly in hanging drops of the type used for tissue culture, by a modification of the technique learnt by the writer at the Strangeways Research Laboratory, Cambridge, to the Director and staff of which he is much indebted.

More than 1,500 larvae have to date been thus isolated and put into more than 200 different media, usually in hanging drops, although some have been tried in Carrel flasks, Petri dishes and other devices. The media have usually been designed to imitate the environment which these parasitic larvae might encounter in the bodies of their hosts and the pH of them has varied from 3·6 to 9·6. In their toleration of this wide pH range these exsheathed parasitic larvae resemble the sheathed infective ones, and this result agrees with the findings of other workers, that parasitic nematodes are remarkably insensitive to pH changes, provided these are reasonably gradual. The parasitic larvae seem, however, not to tolerate a pH below 5 so well as the sheathed infective larvae can. Many of the preparations were daily subcultured, and this seemed, in some instances, to prolong the life of the larvae, although the beneficial effect of it could not be definitely established. The preparations were all kept at

38°–40°C. throughout, except when they were being examined, and then they were not allowed to cool for longer than a few minutes.

Complete details of all the media that have been tried, and of the behaviour of the larvae in them, would require a volume and they cannot be given here. The media were usually used sterile, so that errors due to bacterial action might be eliminated, and the larvae were, of course, sterilised by the Milton solution used to cause their second ecdyses. Even those used for the early experiments, before the method of sterilising the larvae with Milton had been found, were eventually sterilised by washing them after the second ecdysis in sterile water and putting them into sterile media containing an artificial gastric juice composed of Pepsin powder (B.P.) grm. 1, 25% HCl 10 cc., distilled water 500 cc. This formula gives an acidity of 0·5%. In this solution, or after one or two daily subcultures in it, the washed larvae were sterile.

When sterile larvae and sterile media were used bacteria were, however, added to some of them. Among these were the strain of B. coli used for cultivating the infective larvae, other strains of B. coli, B. lactis aerogenes, B. welchii and hay infusion organisms. A cellulose-splitting organism upon which Dr. Stewart, of this Institute, was working (Woodman and Stewart (1928) (1932)), was also used both in the medium in which it was being cultivated and in other media. The larvae lived well in such media.

The parasitic larvae did not feed upon any of the organisms introduced, nor did they show any tendency to do so. So far as the inconclusive results indicated anything, they showed that the larvae were indifferent to all the bacteria used. Much more work is, however, needed, and better control of the experimental conditions, before any conclusion can be drawn about the effect of intestinal bacteria, or of their products upon these larvae. It certainly seems possible that some of these bacteria may affect those adult nematodes which live freely in the intestine, or are loosely attached to it, probably by their effects upon the intestinal contents, rather than by any of their own metabolic products. These adult nematodes probably do not feed upon them, but upon the mucosa, as the work of Hoeppli (1927) and others shows. The bacteria may, however, operate in determining such phenomena as the localisation of certain species to particular regions of the alimentary canal, perhaps doing so chiefly by their effects upon the pH, many species of these bacteria being themselves more or less localised to particular regions of the alimentary canal.

All the media used were prepared by the standard methods given by Hawk and Bergeim (1931), the Medical Research Council's system of Bacteriology (1931) and the workers who invented particular media used. Most of these methods are in use in the media kitchen of this Institute, where the media were prepared. The following is a list of the media and of other substances used either separately or mixed with them :—

Horse serum, sheep serum, the sheep serum water and sheep serum saline recommended by Hogue (1930) for protozoa, whole blood, defibrinated blood with and without $CaCl_2$, blood clot, fibrin, haemoglobin, hematin (Hill and Holden (1926)), peptic digest of blood, pancreatic digest of blood, chick extract, tissue cultures of chick caecum or intestine, starch solid and in solution, dextrose, lactose, maltose, laevulose, glycogen, peptone water, lysin hydrochloride, tryptophane, leucine, tryosine, alanine, glutamic acid, cystein hydrochloride, lecithin, plain broth, serum broth, meat broth, ox bile, sodium taurocholate, saponin, mucin, gastric juice, pancreatic juice, fragments and extracts of sheep abomasum, duodenum or caecum, liver extracts, digests with artificial gastric juice or pancreatic juice of liver, or grass, cultures of cellulose-splitting bacteria with filter paper or with grass, yeast extracts, Meyer's stomach broth, Martin's stomach broth made with either pig or sheep stomach, fat decoctions, tryptic digest of fat, soaps, wheat extract, spinach extract, hay infusion, potassium permanganate, methylene blue, hydrogen peroxide, sodium peroxide, distilled water, tap water, 0·5% ether in water, 5% Milton in water, normal saline, Baker's (1930) medium for spermatozoa, Peters's medium (Lee, 1928), Locke's solution, Pannet and Compton's solution, various kinds of Ringer's solution, solutions of HCl, KCl O_3 and $CaCl_2$.

One of the yeast extracts used was that used by Zimmermann (1921) for the aseptic cultivation of eelworms. It was prepared by Dr. Stewart in this Institute, but usually the larvae died quickly in it, or in serum or other media to which it was added, perhaps because it was too acid. Further experiments are needed, however, with yeast extracts. Glaser (1931, 1932) has successfully cultivated the Oxyurid nematode *Neoaplectana Glaseri*, parasitic in the Japanese beetle *Popillia japonica*, upon agar plates covered with an actively-growing yeast in watery suspension. It does not, of course, follow that nematode larvae parasitic in warm-blooded vertebrates will live well under similar conditions, but the effect of yeast extracts suitably modified might be tried.

The most successful of the inorganic salines used was undoubtedly Peters's medium, in which the larvae will live for several days without osmotic distortion at 38°C., although, of course, they die as soon as the contents of their intestinal cells are exhausted. This medium was, therefore, most frequently used when a diluent for other media was required. It was usually put through a Seitz filter in preference to sterilisation in the autoclave. In a mixture of 3 parts of this and 1 part of serum the larvae lived particularly well and it was in this mixture that the larvae were kept alive longest. They also lived well in a mixture of 1 part of serum with 6 parts of Peters's medium. Another useful medium was the sheep-serum water recommended by Hogue (1930) for protozoa ; this is a mixture of 1 part of serum with 3 parts of distilled water. A similar medium made by diluting 1 part of horse serum with 9 parts of water is also good. Pannett and Compton's saline, used often for tissue cultures, did not give good results, the larvae for some reason dying quickly in it ; and neither Locke's solution, nor any of the modifications of Ringer used, were so good as Peters's medium. This, therefore, or mixtures of this with serum, would probably be the best media to try first for any preliminary experimental work under more accurately controlled conditions ; they could then be modified according to the results obtained.

The writer began using horse serum instead of sheep serum, not merely because it was more conveniently obtained in the Institute, but because there is some evidence that sheep develop a genuine resistance to nematode infections, which may not be merely correlated with their age, but may depend upon factors similar to those which govern immunity and resistance to bacteria. The work of Stumberg (1933) is particularly interesting in this respect, and much more work of this kind is needed, as the summary published by Taliaferro (1929) shows. The writer felt that some of his results with media containing some batches at any rate of the sheep serum used indicated effects which might be those of antibodies in the serum produced by nematode infections of the sheep from which the serum came. It was not possible at the time to go into this point further, and indeed it could not be settled until the actual production of such antibodies is proved beyond doubt ; but it was felt that the use of horse serum would eliminate any such possible factors, because the effects of any present in horse serum would be presumably specific to nematodes infecting

horses and so would not affect the species being used, which are not parasitic in horses.

II. Behaviour of the Parasitic Larvae.

The longest period any parasitic larva was kept alive was 41 days, in a mixture of 3 parts of Peters's medium and 1 part of sheep serum. Some lived more than 30 days in this mixture to which soaps had been added.

In mixtures of 1 part of horse serum and 6 parts of Peters's medium the larvae lived almost equally well; in this mixture when blood clot was added they lived 15–33 days, often disappearing under the clot, so that it could not be decided whether they penetrated into it or not. In Peters's medium alone they lived up to 18 days. In the Locke and methylene blue solution given by Cameron and White (1930), to which 0·2% dextrose was added, they lived 12–14 days.

The addition of lactose, dextrose or laevulose to any of the media made little difference, whether these media were sterile or were inoculated with B. coli, B. lactis aerogenes, or B. welchii. Larvae lived in such media up to 20 days. Dextrose, however, and especially the higher concentrations of it, tended to cause osmotic shrinkage of the larvae; it was especially in media containing this that the distortion of the larvae described below occurred, but a concentration was eventually found in which this distortion could be avoided.

In all the liver extracts the larvae lived only a few days; they seemed to be, like the yeast extracts, toxic to them. None of the media containing vitamins were any better than others used, so that the conclusion of Ackert and his collaborators (1929, 1931a, 1931b, 1931c), based upon experiments with the diet of chickens, that intestinal nematodes of birds do not utilise vitamins as food, was to this extent supported. If vitamins have any effect at all upon nematode infections, they probably act only by increasing the host's resistance (Foster and Cort (1931)).

In peptone water the larvae lived up to 14 days, and as long as this in the chick extract used for tissue culture when it was diluted with Peters's or Pannett and Compton's salines.

In oxalated blood the larvae lived only 3 days or so, and only a day or two when $CaCl_2$ was added to this.

In artificial pancreatic or gastric juices to which fibrin or whole blood clot was added the results were poor. The amino-acids given in the list above were tried separately in Peters's medium and together in various proportions, 1%, 0·5% or weaker solutions being used; they were also added to sera and other media. No evidence was obtained that the larvae lived better in their presence, or, indeed, that they could use any digestive products.

Treatment of the hanging drops with solutions of H_2O_2, or with the vapour of moistened sodium peroxide, usually resulted in vacuolation of the intestinal cells and death of the larvae; and this treatment always failed to revive moribund larvae.

In most of the media tried, however, the larvae lived for 8–10 days and for much longer in the dilutions of serum with Peters's medium, so that there is time for experimental study of them in these. Such studies will undoubtedly suggest media in which it will be possible to cultivate them further.

The movements and the general behaviour of these parasitic larvae were not essentially different from those of the sheathed infective larvae. Usually they did not develop beyond this first parasitic stage, and no increase in size was observed in any of them. In some instances a slight increase in the size of the genital primordium was observed by measurement, but in the majority this did not occur. This absence of signs of growth, however, is not remarkable, because the measurements given by Veglia (1915) and others who have studied these first parasitic larvae in experimental animals show that little or no growth occurs during this stage.

Most of the larvae did, however, form the next sheath preparatory to the next (third) ecdysis. This was often visible as a loose sheath upon them in two or three days in favourable media. The very short tail of this sheath is usually enough to distinguish it from the sheath of infective larvae for which it might easily be mistaken by those who are not familiar with nematode larvae. At this stage also the parasitic larvae are often retracted in their sheaths, as infective larvae so often are, and in drops from which slow evaporation has occurred through the paraffin seal in an incubator, the increased concentration of the medium thus produced may cause further shrinkage of these larvae, so that their sheaths are draped in transverse folds upon them like a stocking on a leg too small

for it. This frequently occurred, for example, in the media containing dextrose, which were the standard media used for the study of bacteria of the colon group or modifications of these. Sometimes a remarkable distension of the intestinal lumen also occurred, so that the intestinal cells were flattened out by it.

The shrinkage and distortion of the whole larva that may thus be produced may, indeed, go so far that the larvae may be not only shrunken and themselves crumpled inside the transversely folded sheaths, but may also be flattened till their bodies are ribbon-like, or the tail end only may be thus flattened while the oesophageal region is swollen, or their whole shape may be so distorted that they are scarcely recognisable and recovery seems impossible. They nevertheless usually remain slowly active, even when they are thus extremely distorted ; and when the drop is opened, and the medium is diluted to its former strength, they slowly resume their normal shape and again fill out the sheath. Many larvae thus extremely distorted have lived for several days after their recovery of their normal outlines. That this is an osmotic effect can be further shown by the direct experiment of increasing the shrinkage and distortion by increasing the salt content of the medium and by the fact that when such shrunken larvae die, their retraction in the sheath disappears, so that they fill the sheath again completely. This was the usual result of the death of larvae in the drops. It may be compared with the same phenomenon seen in infective larvae.

There can be no doubt, therefore, that the life of these larvae, like that of the infective larvae, is governed largely by the permeability of their sheaths, and that the powers possessed by parasitic larvae of recovery from changes due to osmotic forces in their environment are quite as great as those of the infective larvae. These powers must have very great survival value, and it is unlikely that any sound method of cultivating these larvae further will be found until the permeability of their sheaths is understood.

Although most of these larvae thus reached the stage immediately preceding the third ecdysis, only 10 out of more than 1,200 studied actually performed this ecdysis. The others died with the sheaths intact, and no method was found by which it could be artificially removed. The 1 in 20 dilution of Milton used for the production of the second ecdysis had no power to produce the third ecdysis even when the larvae were first washed

in sterile distilled water to remove any organic matter that might be interfering with its action. It seemed, indeed, to have little effect on the larvae at all. The effect of oxygen was tried on some of them, but its only effect was to cause the vacuolation just described.

The ultimate fate of most of the larvae was, in fact, the same. They lived for a longer or shorter period in a healthy state, and then became less active. Subsequently their intestinal cells and oesophageal region began to show vacuoles, and it could be seen that the intestinal cells were slowly emptying and that their granular contents were being used up. The whole body of the larvae looked more transparent, and eventually, if they lived long enough, they became little more than strings of vacuoles, and, finally, they died inside their sheaths. It was judged after a long series of experiments, that daily subculturing of the isolated larvae might sometimes prolong their lives ; but this was by no means certain. The general impression obtained was that the factor lacking was not oxygen, nor any particular substance which frequent renewal of the medium might supply, nor even removal of excretory products, but a better understanding of the physical factors by which their physiology is governed. In view of the fact stated by many workers that lactic and fatty acids are usually present in the environment in which adult nematodes are living, some drops in which larvae had been living for many days without subculture were tested by Dr. Stewart for lactic and fatty acids, but no evidence of their presence was obtained. The amount of fluid available, was, however, very small and little importance was attached to this result.

III. The Third Ecdysis.

When this was performed by the 10 larvae in which it was observed, it usually occurred in the manner described and figured by Veglia (1915), namely by a rent in the sheath at the side of the oesophageal region, about halfway down the oesophagus, and not by the detachment of the cap which is so characteristic of the second ecdysis. The invagination of the anterior end of the sheath by the emerging head of the fourth larva, which Veglia (1915) describes, was not seen nor were the fourth larvae seen, as Veglia saw them, swimming about with fragments of this anterior end of the sheath still attached to them. All the larvae which performed the third ecdysis in the writer's preparations died almost as soon as they emerged. This may have been due to

the acidity of the media in which these third ecdyses occurred, which usually contained gastric juice.

The occurrence of these third ecdyses did not, however, appear to be related to the composition of the media in which they occurred, because the majority of larvae studied in all these media did not effect the third ecdysis. It is true, however, that 6 of the 10 third ecdyses observed occurred in a mixture of equal parts of gastric juice and sheep serum water after 4–9 days, usually with a daily subculture into fresh medium. One occurred after 15 days in a mixture of equal parts of sheep serum water and yeast extract prepared by Dr. Stewart in the Institute according to the formula given by Zimmermann (1921), and one in a solution containing bleaching powder. This last was the only instance in which any hypochlorite solution appeared to affect the third ecdysis and the larva was not observed between the 8th and the 20th day in it, when it was found dead and half out of its sheath. The other two third ecdyses are described below.

Nor did there seem to be any relation of these third ecdyses to the fluid in which the second ecdysis of the larvae undergoing them had occurred. This had happened in three instances in artificial pancreatic juice; the others had occurred respectively in 1% Na_2CO_3, equal parts of gastric juice and sheep serum water, yeast extract and sheep serum water, 1 in 20 Milton and the above-mentioned bleaching powder solution.

IV. Simultaneous Second and Third Ecdyses.

In two instances the remarkable phenomenon occurred of the simultaneous occurrence of the second and third ecdyses. This occurred, that is to say, in larvae whose outermost sheath, *i.e.* that of the infective or third larva, enclosed a fourth larva which was also loose in its sheath, *i.e.* the separated skin of the third larva. Both these sheaths were cast at the same time, that is to say, the fourth larva broke its way out of both together. No explanation of this phenomenon can be offered. It occurred in one instance after 15 days in a mixture of gastric juice and mucin. In the other instance it occurred after 7 days in a mixture of 3 parts of Peters's medium, 1 part of a glycogen solution and 1 part of peptone water. In both instances the fourth larva was dead practically as soon as it emerged.

Such a phenomenon may seem at first sight obviously abnormal, but it must be remembered that infective larvae can frequently be seen, either in faecal or bacterial cultures, with the sheath already loose upon the larvae inside them; in other words, the third lethargus stage, during which the third larva separates its sheath preparatory to the third ecdysis, may occur before the second ecdysis has occurred. Such larvae are not at all uncommon in faecal and bacterial cultures, and there seems to be no reason why they should not occur in nature. If they do, we must conclude that some larvae, and especially those which have long been upon the pastures, may not succeed in entering the host until they are in reality at the fourth stage, when they are much more dependent upon the conditions governing parasitic life than the third stage is. It is possible, therefore, that if they first emerged inside the host at this stage, they might not be infective, because they would have been deprived of the adaptational value of the third larval stage.

V. Food of the Larvae, their relation to Bacteria and their Variable Viability.

Little reliable information was gathered from these experiments about the physiological needs of these parasitic larvae. The attempts made to study their respiration, and to find out whether they sought any elements of the blood for this purpose were not completed. Nor was any information gained about the food, nor their method of feeding. We have, indeed, no certain knowledge that they feed at all. They cannot of course, feed after the separation of their sheaths has cut them off from their environment. That they feed in some way on the mucosa of the host, or on the blood clots under which, according to Veglia (1915), they lie and which their presence in some way provokes, seems unlikely, because when blood clot, fibrin, or fragments of the mucosa of the stomach or duodenum or caecum of sheep were introduced into the writer's preparations, these larvae did not always burrow into them, nor did they live as well in such preparations as in others without these additions. When they burrowed into these fragments they usually died inside them, or remained entangled in the mucus at the edge of the mucosa fragments. They did not burrow into fragments of 2% watery agar, and no definite thigmotactic, nor chemotactic reaction towards tissue fragments was observed, nor did they react differently in tissue cultures containing living duodenum of the

chick. If, as seems likely, they do not grow during this stage, it is possible that they do not feed, but rely upon the stores in the granular contents with which their intestinal cells are packed, the exhaustion of which usually heralds their death.

The fact, however, shown by the writer and described in another paper (1933a), that these parasitic larvae can carry in their intestinal lumen bacteria taken in before they are shut off from their environment by the separation of the loose sheath of the infective larva, opens up, not only the possibility that they could thus inject such bacteria directly into the tissues of the host—a possibility which would be important if such bacteria were pathogenic—but also the possibility that they feed on these retained bacteria during the first parasitic stage, as well as on the stores in the intestinal cells. This point needs further investigation.

One point may be also mentioned, because it merits the further attention of experimental workers and may have some bearing upon the possibilities of infection. It was repeatedly observed that some larvae die quickly in media in which others from the same culture lived for many days. This variation in the vitality of individual larvae may not be noticed unless they are isolated and studied individually. It is also characteristic of infective larvae, and it may be equally true of the eggs. It seems especially true of both infective larvae and parasitic ones taken from faecal cultures and is, therefore, all the more likely to be true of these larvae developing in nature. When it is seen in parasitic larvae it is probably not an effect of the Milton exsheathing solution upon them, because experiment showed that the majority of larvae exsheathed in this solution will live in it for 3 or 4 days at 39°C. and because it was noticed only in some of the larvae exsheathed in this solution ; these were discarded, and the more than 1,500 used showed no bad effects of the exsheathing solution. It is therefore more probably due to the effect of the environment on the stages preceding the infective stage. Whether improved methods of cultivating infective larvae on pure cultures of bacteria, which may result from experimental work upon the methods at present available, will provide us with larvae of more uniform vitality, remains to be seen.

These variations in their vitality should, however, be taken into consideration when animals are being experimentally infected with infective and with parasitic larvae. It by no means follows that, if two animals are given 10,000 larvae each, the resulting infection can be regarded as equal

in both. The resistance of the animal, whatever that may turn out to be, and various other factors must also be considered, as well as the variable viability of the larvae. Yet this assumption is commonly made, just as the admittedly only approximate results of egg counts are used as a basis for experiments from which important conclusions are drawn.

The failure of all these attempts to cultivate the larvae further was disappointing, but it was at least learned that such attempts would be more likely to succeed if the third ecdysis could be artificially induced, because the failure seemed to be largely due to the absence of third ecdyses. The writer therefore decided to study the second ecdysis further in the hope that an understanding of this might throw light upon the factors governing all the ecdyses. The results of the study of the second ecdysis (Lapage (1934)) have not yet been applied to the third ecdysis; but this will be done.

Meanwhile, the other necessity is more knowledge of the physiology of the first parasitic larvae, which can now be studied in any sterile media required, because, until we have this, we shall not be able to cultivate under favourable conditions any fourth parasitic larvae which experimental control of the third ecdysis may provide for study.

Summary.

1. Attempts were made to cultivate in sterile artificial media the sterilised first parasitic larva of intestinal nematodes of sheep. These larvae were obtained by artificial production of the second ecdysis in 1 in 20 dilutions of Milton hypochlorite in distilled water.

2. Over 1,500 larvae were thus isolated, usually in hanging drops, in more than 200 different sterile media, containing ingredients likely to be present in the normal environment of these larvae inside their hosts.

3. None of the larvae showed any growth. Most formed the next sheath and were ready for the third ecdysis, but only 10 larvae actually performed this. The parasitic third larva thus liberated always emerged by a rent at the side of the oesophageal region of the sheath, and never by detachment of a cap like that characteristic of the second ecdysis. In every instance the parasitic third larva died immediately after the third ecdysis, which set it free. Two of these 10 larvae underwent the second and third ecdyses simultaneously.

4. The methods used by the writer (1933b) to induce artificially the second ecdysis always failed to produce the third ecdysis. No method of producing this at will was found.

5. The longest time any first parasitic larva lived was 41 days. Few of them lived, however, less than 8–10 days. A life of 18–30 days was more usual before visible signs of physiological abnormality appeared, such as the gradual vacuolation and emptying of the intestinal cells which usually preceded their death.

6. Those which were ready with a loose sheath for the third ecdysis, showed, as infective larvae also do, remarkable powers of resistance to changes produced in them by osmotic factors.

7. None of the larvae showed any particular reaction to blood, mucosa of the stomach or duodenum, nor, indeed, to any of the ferments or tissues these larvae encounter in their hosts. They seemed to be as indifferent in this respect as the sheathed infective larvae are.

8. A comparative physiological study of the sheathed infective and the exsheathed first parasitic phases of the second larva would verify, and perhaps modify, our knowledge of the functions of the so-called protective sheath of the infective larva.

References.

Ackert, J. E. & Spindler, L. A., 1929.—" Vitamin D and resistance of Chickens to Parasitism." *Amer. J. Hyg.*, 9, 292–307. (*W.L.* 600a.)

Ackert, J. E. & Beach, T. D., 1931a.—" Yeast as a Factor in the Growth of the Fowl Nematode. Ascaridia lineata (Schneider)." *Jl. Parasit.*, 18, 113. (*W.L.* 11425.)

Ackert, J. E. & Nolf, L. O., 1931b.—" Resistance of Chickens to Parasitism affected by Vitamin B." *Amer. J. Hyg.*, 13, 337–344. (*W.L.* 600a.)

Ackert, J. E., McIlvaine, M. F. & Crawford, N. Z., 1931c.—" Resistance of Chickens to Parasitism effected by Vitamin A." *Amer. J. Hyg.*, 13, 320–336. (*W.L.* 600a.)

Baker, J. R., 1930.—" A Fluid for Mammalian Sperm-suspensions." *Quart. J. exp. Physiol.*, 20, 67–70. (*W.L.* 17502.)

Cameron, A. T. & White, F. D., 1930.—" A Course in Practical Biochemistry." *London: J. & A. Churchill.*

Foster, A. C. & Cort, W. W., 1931.—" The Effect of Diet on Hookworm infestation in Dogs." *Science*, 73, 681–683. (*W.L.* 19938.)

Glaser, R. W., 1931.—" The Cultivation of a Nematode Parasite of an Insect." *Science*, 73, 614–615. (*W.L.* 19938.)

——, 1932.—" Studies on Neoaplectana Glaseri, a Nematode Parasite of the Japanese Beetle (Popillia japonica)." *State of New Jersey Dept. of Agric., Circular* No. 211, 3–34.

HAWK, P. B. & BERGEIM, O., 1931.—" Practical Physiological Chemistry." 10th Edn. *London : J. & A. Churchill.*

HILL, R. & HOLDEN, H. F., 1926.—" The Preparation and some properties of the globin of Oxyhaemoglobin." *Bio-chem. J.*, 20, 1327–1339. (*W.L.* 2967.)

HOEPPLI, R., 1927.—" Ueber Beziehungen zwischen dem biologischen Verhalten parasitischer Nematoden und histologischen Reaktionen des Wirbeltierkörpers." *Arch. Schiffs- u. Trop. hyg., Beihefte zum Bd.*, 31, 203–290. (*W.L.* 1804.)

HOGUE, M. J., 1930.—In Hegner, R. W. & Andrews, J., 1930.—" Problems and Methods of Research in Protozoology." *New York : The MacMillan Co.*

LAPAGE, G., 1933a.—" The Cultivation of Infective Nematode Larvae on Pure Cultures of B. Coli." *3rd Rep. Dir. Inst. of Animal Path., Univ. of Cambridge*, 237–270.

——, 1933b.—" Cultivation of Parasitic Nematodes." *Nature*, 131, 583–584. (*W.L.* 14900.)

——, 1935.—" The Second Ecdysis of Infective Nematode Larvae." *Parasitology*. 27, 186–206. (*W.L.* 16035.)

LEE, Bolles, 1928.—" Microtomist's Vade-Mecum." 9th Edn. Edited by Gatenby, J. Bronte, Cowdry, E. V. and collaborators. *London : J. & H. Churchill.*

MEDICAL RESEARCH COUNCIL, THE, 1931.—" A System of Bacteriology in Relation to Medicine." 9 Vols. by various authors. *London : H.M. Stationery Office.*

STUMBERG, J. E., 1933.—" The Detection of the Proteins of the Nematode Haemonchus contortus in the Sera of Infected Sheep and Goats." *Amer. J. Hyg.*, 18, 247–265. (*W.L.* 600a.)

TALIAFERRO, W. H., 1929.—" The Immunology of Parasitic Infections." *New York & London : The Century Co.*

VEGLIA, F., 1915.—" The Life History and Anatomy of Haemonchus contortus (Rud)." *3rd & 4th Rep. Dir. Vet. Res., Union of S. Africa*, 347–500.

WOODMAN, H. E. & STEWART, J., 1928.—" The Mechanism of Cellulose Digestion in the Ruminant Organism. II. The transformation of Cellulose into glucose by the agency of Cellulose-splitting Bacteria." *J. agric. Sci.*, 18, 713–723. (*W.L.* 10966.)

——, 1932.—" The Mechanism of Cellulose Digestion in the Ruminant Organism. III. The action of Cellulose-splitting Bacteria on the Fibre of certain typical feeding stuffs." *Ibid.*, 22, 528–547.

ZIMMERMANN, A., 1921.—" Researches expérimentales sur l'élevage aseptique de l'Anguillule du vinaigre Anguillula oxophila Schneider." *Rev. suisse Zool.*, 28, 357–380. (*W.L.* 19288.)

Journal of Helminthology, Vol. XIII, *No.* 2, *May*, 1935, *pp.* 129–138.

On *Heterodera schachtii*, with special reference to the Oat race in Britain.

By E. E. Edwards, M.Sc.

(*Advisory Zoologist, University College, Cardiff; formerly Adviser in Agricultural Zoology, Harper Adams Agricultural College, Newport, Shropshire.*)

Introduction.

In August 1933, Mr. W. Usher, Assistant Agricultural Organiser for Shropshire, called the writer's attention to a partial failure of spring oats at Montford Bridge, Shrewsbury. The Advisors in Agricultural Chemistry and Mycology for the West Midland Province had previously inspected the crop and had failed to find evidence of any manurial deficiency or fungal attack likely to be responsible for the failure. A careful examination of the roots of unthrifty plants by the writer, however, showed that they were heavily attacked by *Heterodera schachtii* in all stages of development. Further observations revealed that although the cysts of this eelworm were present on the oats over the whole field, very little disease was apparent on the plants except on those growing in certain " sick " areas. The plants in these affected patches were very much stunted, with small ears and shrivelled grain, the foliage was pale in colour and the lower leaves in most cases dead, the roots were excessively branched and rather heavily infested with *H. schachtii*. It would appear that the infection by the eelworm in the present instance is one of long-standing since cereal crops, particularly oats, had been almost a complete failure for some years in these " sick " areas. It is also significant that the infested areas which were of varying size had in recent years, according to the farmer, greatly increased in extent and that with each successive oat crop new affected patches had made their appearance. In view of this apparent old-standing infection, a special search was made for the presence of *H. schachtii* on cereal crops in other fields in the neighbourhood. It soon became evident in the course of this preliminary search that *H. schachtii* was already well established on several farms in the district and that besides oats, wheat and barley were also subject to infestation.

The investigation was continued the following year when all cases of failing cereal and other cultivated crops reported in Shropshire were examined. In addition, a thorough examination was also made of the roots of apparently healthy looking crops in the immediate neighbourhood of those failing ones that were found infested with *H. schachtii*. Later in this paper the general field observations made during this investigation are described, together with an account of some cultural experiments that were carried out with a view of determining whether the race of *H. schachtii* found parasitic on cereals in Shropshire is capable of attacking other plant species of economic importance and whether this race is distinct from those commonly found in the County on potatoes and peas, respectively.

History and Distribution of the Cereal Strain of *H. schachtii*.

Heterodera schachtii, Schmidt, 1871, first discovered and studied by Schacht in 1859 was recognised as a parasite injurious to cereals by Kuhn in 1874. Since then, this race of *H. schachtii*, highly specialised to oats and to a less extent to barley, wheat and rye, has become very widespread and injurious to these crops in Germany, Holland, Norway, Sweden and Denmark (Goodey—1933). Recently this eelworm has also been found severely damaging oats and wheat in Australia (Hickinbotham, Davidson—1930).

In Britain the first records of *H. schachtii* on cereals are those by Theobald who reported the parasite on oats from Lincolnshire in 1908 and on wheat from Kent in 1909. No subsequent attacks on cereals seem to have been recorded until 1933 when " a severe infestation on oats associated with partial failure of crop in a 25 acre field near Shrewsbury " was reported by the writer. In 1934 Miles discovered " extensive local injury to oats on light, loamy soil near Altrincham ; field reported to do ' oats badly ' : potatoes in same field unattacked."

Symptoms of " Oat-Sickness."

The condition termed " oat-sickness " and which is now found to be due to, or, at least, associated with *H. schachtii* attack, is characterised by patches of plants in the field presenting a general unthrifty and unhealthy appearance. At first the plants grow normally but towards the middle of May when they have reached the 3- or 4-leaf stage, the

leaves turn slightly red at the edges as though the plants suffer from deficiency of available potash in the soil. As the season advances, the foliage of affected plants becomes pale green in colour and the apical margins of the leaves gradually turn yellow. This discoloration develops more pronounced in time and the plants when an attack is severe either die or at the best make only poor growth. Even when the plants attain maturity, the ears remain small and frequently consist of unfertilised flowers and shrivelled grain. The root system is also deformed and much shallower than that of normal plants. The main roots instead of being long and almost unbranched are short, abnormally thickened and with excessive development of lateral rootlets. If examined at the appropriate time of the year the encysted females of *H. schachtii* will also be seen attached to the roots.

General Field Observations.

The following general field observations on the cereal strain of *H. schachtii* cover those made of infested areas in Shropshire during 1933 and 1934 and must be regarded as of an essentially preliminary nature.

In distribution this race of *H. schachtii* is very widespread in many localities in Shropshire, particularly over an area extending for a radius of fifteen miles from Shrewsbury. In all cases examined where crop had completely or partially failed and where the eelworm cysts were abundant, it was found that cereals had been grown frequently in the rotation for a very long period. In most of these instances, cereals had been grown for several successive years or had formed at least two of the crops in the four course rotation which is commonly practised in the area.

There can be little doubt that several districts in Shropshire harboured infection of the cereal strain of *H. schachtii* long before the diagnosis of the parasite at Montford Bridge in 1933, for several farmers had for some years previously noted the development of " oat-sickness " on their land. The account given by these farmers in Shropshire of the development of " oat-sickness " on their holdings is essentially similar to that of " potato-sickness " or " beet-weariness " (Rübenmudigket). At first the " oat-sickness " involved but few isolated " bad patches " which with frequent cropping of the land with cereals gradually increased in area and, in extreme cases, merged into one another until whole fields became affected and thereby unfit for grain-production.

Different varieties of cereals vary in the degree to which they are liable to infestation as judged by the intensity of the diseased symptoms now associated with the presence of *H. schachtii* on the roots of the plants. Oats seem to suffer far more regularly and severely than other cereals, though both wheat and barley may occasionally be seriously affected. Further, autumn sown crops appear to be definitely more resistant to injury than those sown in the Spring. No rye is grown in the County and no observations were therefore possible on this crop.

Although actual eelworm cyst counts of the different affected areas examined were not carried out in the course of these preliminary field studies, it would seem from the various visual observations made that *H. schachtii* may not be the only factor in the causation of what is termed " oat-sickness " of cereals. It was often noted that apparently healthy plants frequently showed heavy infestations of *H. schachtii* cysts on the roots without suffering any conspicuous damage or obvious diminution of the crop yield. Furthermore, in a great many cases, cysts appeared to be equally common on the healthy looking cereals as on those in the same field but growing in " bad patches " and where the pathological condition in the plant was most pronounced. On the other hand, several instances were observed where there seemed to be a positive correlation between the number of female worms attached to the roots and the state of the crop with regard to manifestation of eelworm disease. Again, all cases of failing crops showing symptoms of " sickness " were found invariably accompanied by heavy infection of *H. schachtii*.

Pot Experiments.

The present experiments were conducted in 1933 as already described, with a view of ascertaining whether the strain of *H. schachtii* found parasitic on cereals in Shropshire is capable of attacking other plant species of economic importance and whether it is a distinct race from those commonly found in the County on potatoes and peas, respectively. Both the potato and pea strains of *H. schachtii*, associated with " potato sickness " and " pea sickness," respectively, were, at this date, well established in several localities in Shropshire. While little experimental data was available at the time to provide evidence in favour of the acceptance of the theory of two distinct biological races of *H. schachtii* in this County, general field observations carried out during the previous three years certainly indicated the existence of at least a very marked

preference for some particular hosts. In no instance had it been noted for potatoes to become infested with *H. schachtii* when grown on " pea sick " ground or alternatively for an attack of this eelworm to develop on peas when grown on " potato sick " land. Crops of peas and potatoes had on numerous occasions been observed growing side by side with one of the crops heavily infested with cysts of *H. schachtii* and the other entirely free of infestation. Of these two races, the potato one appeared to be highly adapted and capable of living on one host only, namely, the potato, though a single instance of slight infection had been noticed in 1931 on tomatoes in a private garden at Dorington. The pea strain, on the other hand, seemed to be less specialised and able to parasitize, in addition to culinary peas, a variety of other host plants but principally representatives of the natural order *Leguminosae*. Amongst the latter, peas, broad beans, vetches and red clover had been found frequently infected, while of the plants of the other natural orders, mustard and radish only were known subject to attack and these but to a very slight degree and on very rare occasions.

For the trials already mentioned, three series of pot experiments were set up in which a variety of vegetables, cereals, grasses, clover and other leguminous plants were grown in soil known to be heavily infested with :—

Series I.—" Potato race " of *Heterodera schachtii*.
Series II.—" Pea race " of *H. schachtii*.
Series III.—" Oat race " of *H. schachtii*.

Each series contained two pots of each of the fifty-two species of plants tested. All the plants were grown from seed sown directly in the pots with the exception of cabbages, cauliflowers, broccoli, brussel sprouts, tomatoes and celery, which were transplanted in the seedling stage into the pots, having previously been raised in sterilised soil. All the pots were sown or planted up in the third week in April. They were then plunged in groups, according to their series, in a bed of ashes and soil in an open air wire-covered enclosure, being carefully watered throughout the season in such a way as to approach field conditions as closely as possible.

One pot of each of the fifty-two species of plants in each series was turned out towards the middle of July when the roots were carefully examined for cysts of *H. schachtii*. Similar inspection was made of the

remaining plant of each species in each of the three series during the latter part of September.

The fifty-two species of plants tested in this way for cystic females of *H. schachtii* were :—

1. Wheat (*Triticum vulgare*).
2. Oat (*Avena sativa*).
3. Barley (*Hordeum sativum*).
4. Rye (*Secale cereale*).
5. Perennial Ryegrass (*Lolium perenne*).
6. Italian Ryegrass (*Lolium italicum*)
7. Tall Fescue (*Festuca elatior*).
8. Meadow Fescue (*Festuca pratensis*).
9. Rough Stalked Meadow Grass (*Poa trivalis*).
10. Smooth Stalked Meadow Grass (*Poa pratensis*).
11. Annual Meadow Grass (*Poa annua*).
12. Cocksfoot (*Dactylis glomerata*).
13. Meadow Foxtail (*Alopecurus pratensis*).
14. Timothy (*Phleum pratense*).
15. Crested Dogstail (*Cynosurus cristatus*).
16. Golden Oat Grass (*Avena falvescens*).
17. Sweet Vernal Grass (*Anthoxanthum odoratum*).
18. Red Clover (*Trifolium pratense*).
19. Crimson Clover (*Trifolium incarnatum*).
20. Wild White Clover (*Trifolium repens*).
21. Alsike (*Trifolium hybridium*).
22. Trefoil (*Medicago lupulina*).
23. Sainfoin (*Onobrychis sativa*).
24. Lucerne (*Medicago sativa*).
25. Vetch (*Vicia sativa*).
26. Tall Pea (*Pisum sativa*).
27. Dwarf Pea (*P. sativa*).
28. Sweet Pea (*Lathyrus odoratus*).
29. Broad Bean (*Faba vulgaris*).
30. Dwarf Bean (*Phaseolus vulgaris*).
31. Runner Bean (*Phaseolus multiflorus*).
32. Cabbage (*Brassica oleracea capitata*).
33. Brussel Sprouts (*Brassica oleracea bullata gemmifera*).
34. Broccoli (*Brassica oleracea botrytis asparagoides*).
35. Cauliflower (*Brassica oleracea botrytis cauliflora*).
36. Swede (*Brassica rutabaga*).
37. Mustard (*Brassica alba*).
38. Radish (*Raphanus sativus*).
39. Lettuce (*Lactua sativa*).
40. Potato (*Solanum tuberosum*).
41. Tomato (*Lycopersicum esculentum*).
42. Garden Beet (*Beta vulgaris*).
43. Sugar-Beet (*Beta vulgaris*).
44. Mangold (*Beta vulgaris*).
45. Spinach (*Spinacia oleracea*).
46. Leek (*Allium porrum*).
47. Onion (*Allium cepa*).
48. Carrot (*Daucus carota*).
49. Parsnip (*Pastinaca sativa*).
50. Parsley (*Carum petroselinum*).
51. Celery (*Apium graveolens*).
52. Strawberry (*Fragaria vesca*).

Series I. (*Potato race.*)

Of all the different varieties of plants grown in the potato-sick soil known to be thoroughly infested with *H. schachtii* (Series I), no cysts of this eelworm were found on the roots of any of them except on those of the potato. The roots of the latter, as was to be expected, were thickly covered with cystic females and the haulm completely died down early in the season. It would appear from these experiments and the general field observations already described that *H. schachtii* commonly found on the potato in Shropshire is, as in other parts of Great Britain, so specialised on this host plant as to form a distinct biological strain capable of attacking under ordinary conditions a single host species only though occasionally the tomato plant may become slightly infected. It must be emphasised, however, that although this race of *H. schachtii* seems to have lost the power of attacking other plants of economic importance it is by no means certain that such power might not be regained in the event of the repeated growing of some crop other than the potato in an infected area.

Series II. (*Pea race.*)

Examination of the roots of the various plants grown in the "pea-sick" soil (Series II) revealed that cysts of *H. schachtii* were present in the case of both the tall and dwarf pea, broad bean, vetch, red clover, potato, radish and mustard. Amongst these leguminous plants, there was a marked difference in the intensity of attack by this nematode, judging by the number of cysts on the roots; tall and dwarf peas were the most severely parasitized, and the red clover the least, whilst the broad beans and vetches were intermediate in this respect. In contrast to the leguminous plants, the potato, radish and mustard were found, on the other hand, to bear but a very slight infection, the maximum number of cysts being seven on one of the radish plants.

Despite the presence of heavy infestation of female worms, no noticeable pathological condition was evident on the leguminous plants except on the peas, which were obviously adversely affected. In this case the plants were greatly dwarfed, pale in colour, and died off at an early stage. It is significant, however, that these infested peas were also rather severely attacked by the root fungus—*Aphanomyces euteiches* and that no case of heavy infection of *H. schachtii* on the pea has ever been seen by the

writer in the course of extensive observations in Shropshire and Staffordshire free from an infection of this fungus or *Thielavia basicola* either singly or together. Incidentally it may be noted that presence of a fungus —*Fusarium* sp.—was also a constant feature in similar outbreaks recorded by Triffitt from the counties of Glamorgan and Worcester during 1931. These experiments taken by themselves strongly suggest that the degree of specialisation which is shown by the race of *H. schachtii* on peas in Britain is not of such high order as that which is exhibited by the well-known strain of this parasite on potatoes. Further, that this degree of specialisation of the pea race varies in different parts of the country since the one associated with " pea-sickness " in the Evesham district of Worcestershire failed to infect, or, at least, attain sexual maturity on, potatoes in the susceptibility trials, carried out by Walton, Ogilvie and Mulligan in 1933.

The possibility is not excluded of the occurrence, however, of two different races in the area from which the soil for the present experiment was obtained, one able to attack potatoes and the other peas. Nevertheless, the more feasible explanation at the moment seems to be that the pea race had succeeded in going over to the potato, but further investigations are needed to elucidate this point. No such comparison is possible with regard to mustard and radish as these plants were not included in the trials conducted by these three workers.

Series III. (*Oat race.*)

In the case of the plants grown in the " oat-sick " soil (Series III), all the cereals showed the presence of cysts of *H. schachtii* on their roots. The heaviest infestation occurred on the oats, though barley and wheat were also rather seriously infected. Rye, on the other hand, was comparatively free from attack and in contrast to the other three cereals made a luxuriantly healthy growth throughout the growing season. In addition to cereals, the oat race of *H. schachtii* was found to have attacked both Perennial and Italian ryegrasses, as well as red clover, and had been able to reach sexual maturity, at least, on the former grass almost as readily as on the oat, judging from the number of ripe females attached to the roots. Although Italian ryegrass and red clover were extensively parasitised they showed a decidedly stronger immunity to infestation than either perennial ryegrass or any of the cereals with the exception of rye. That the other grasses and leguminous plants gave negative

results in these experiments is not taken as conclusive evidence of their immunity, since this could only be established by further extensive trials, under natural conditions.

These susceptibility trials carried out in soil from an "oat-sick" area, give definite indications that the race of *H. schachtii* on cereals in Shropshire is able to attack other plant species of economic importance and that the race is distinct from those commonly found in this county on potatoes and peas, respectively. It is realised, however, that the exact range of host plants liable to attack by this or any other parasite cannot be definitely established from the results of a single year's experiments and that further extensive trials must be conducted over several seasons before final conclusions can be reached.

Control Measures in Relation to Crop Rotation.

The evidence presented in this paper points to the conclusion that the potato race of *H. schachtii* in Shropshire, is, as in other parts of Britain, so highly specialised to this particular plant that any other common agricultural or horticultural crop with the exception of the tomato can be grown on "potato-sick" land without risk of infection. In view, however, of the apparent attack of the potato race on the carrot recorded by Triffitt from Wiltshire in 1931, such host plant should not be included in crop rotations designed for the control of this parasite.

The pea race also seems to be so highly adapted that, with the exception of broad beans, culinary peas, vetches and red clover, almost any other crop might be successfully grown and without risk of infestation if the land, from other points of view, is suitable for their cultivation. In planning suitable crop rotations for exterminating the nematode entirely from infested land, the inclusion of potatoes, mustard and radish should also be avoided since these three crops appear to be slightly susceptible to attack and would therefore provide means for the perputation of the parasite.

Great practical difficulties are involved in planning suitable crop rotations whereby the oat race can be controlled as this strain is capable of attacking and reproducing freely not only on cereals but also on the ryegrasses and red clover. Infested land cannot be returned to pasture for several years with the object of eradicating the soil completely of the eelworm, for perennial ryegrass, even when omitted in the seed mixture,

invariably in most districts becomes established very shortly in the sward. The grass and clover ley for such land must therefore be one of short duration and neither of the ryegrasses (*Lolium* spp.) nor red clover (*Trifolium pratense*) included in the seed mixture. Where frequent cropping with a cereal crop cannot be avoided a resistant variety of barley, e.g., *Chevalier* (Goodey 1933) should be selected.

References.

Davidson, J., 1930.—" Eelworms (Heterodera schachtii Schm.) affecting Cereals in South Australia." *J. Dep. Agric. S. Aust.*, xxxiv, 378–385. (*W.L.* 11154.)

Edwards, E. E., 1933.—Ministry of Agriculture and Fisheries, Monthly Summary of Plant Pests and Diseases, September.

Goodey, T., 1933.—Plant Parasitic Nematodes, pp. 128, 144, 146 and 147.

Hickinbotham, A. R., 1930.—" Eelworm and No-growth Patches." *J. Dep. Agric. S. Aust.*, xxiv, 386–392. (*W.L.* 11154.)

Kuhn, J., 1874.—" Ueber das Vorkommen von Rübennematoden, an den Wurzeln der Halmfrüchte." *Z. Ver. Rubenzucker Ind. Zollver.*, xxiv, 149–153.

Miles, H. W., 1934.—Ministry of Agriculture and Fisheries, Monthly Summary of Plant Pests and Diseases, August.

Schacht, H., 1859.—" Ueber einige Feinde und Krankheiten der Zuckerrube." *Z. Ver. Rubenzucker Ind. Zollver.*, ix, 390.

Schmidt, A., 1871.—" Ueber den Rübennematoden (Heterodera schachtii.) " *Z. Ver. Rubenzucker Ind. Zollver.*, xxi, 1–19.

Theobald, F. V., 1908.—"An attack of *Heterodera* in Oats." *J. S.-E. agric. Coll., Wye.*, xvii, 150–151. (*W.L.* 11557.)

——, 1909.—" Wheat attacked by *Heterodera*." *J. S.-E. agric. Coll., Wye.*, xviii, 142. (*W.L.* 11557.)

Triffitt, M. J., 1931.—" On the Eelworm *Heterodera schachtii* attacking Peas in Britain." *J. Helminth.*, ix, pp. 175–177. (*W.L.* 11224b.)

——, 1931.—" On the Occurrence of *Heterodera radicicola* associated with *Heterodera schachtii* as a Field Parasite in Britain." *J. Helminth.*, ix, pp. 205–208. (*W.L.* 11224b.)

Walton, C. L., Ogilvie, L. and Mulligan, B. O., 1933.—" Observations on the Pea Strain of the Eelworm *Heterodera schachtii* and its relation to Pea-Sickness." Long Ashton (Bristol) Ann. Rep., pp. 74–85.

Journal of Helminthology, Vol. XIII, *No.* 3, *July*, 1935, *pp.* 139–148.

Some Helminth Parasites from Partridges and other English Birds.

By Phyllis A. Clapham, Ph.D.

(*Lawrence Research Student of the Royal Society.*)

(*From the Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

During two years, while collecting helminth material for dietary experiments, a large number of game birds were made available to me at the Institute of Agricultural Parasitology, St. Albans, through the courtesy of Mr. A. D. Middleton of the Bureau of Animal Population, University Museum, Oxford. These were handed over to the present writer by Dr. R. T. Leiper, F.R.S., for post-mortem examination. The birds were sent in as they died from a large number of estates in the following counties—Bedfordshire, Berkshire, Buckinghamshire, Cambridgeshire, Cheshire, Derbyshire, Hampshire, Herefordshire, Hertfordshire, Norfolk, Northamptonshire, Northumberland, Nottinghamshire, Shropshire, Suffolk, Sussex, Worcestershire and Yorkshire, and hence it can be assumed that the helminths obtained are a representative sample of those parasitizing English partridges. The following notes are obtained from the records of the post-mortem examinations.

A total of 380 birds has been examined of which 131 were shot for experimental purposes, leaving 249 which died as a result of disease. There were, however, 108 birds which died 3 days or less after hatching and therefore showed no signs of helminthic infestation.

Of the total number of birds examined, 205 were found to be infested with helminth parasites, many having more than a single species.

142 birds contained 1 species of helminth.
46 ,, ,, 2 ,, ,,
13 ,, ,, 3 ,, ,,
4 ,, ,, 4 ,, ,,

Only in 27 cases could death be attributed to helminthic infestation. The other birds had died as a result of a variety of causes with which the

present report is not concerned. Gapes were responsible in 15 cases, all of which were young birds. Strongylosis (*Trichostrongylus tenuis*) was responsible in 11 cases and the cestode *Davainea proglottina* had caused the death of a single bird. The low percentage of deaths due to strongylosis may be commented upon as this disease has often been reported as being very prevalent and the cause of severe losses. The disease in grouse was indeed the subject of an intense investigation about 1910, the results of which were published and edited by Leslie and Shipley. In partridges it was reported upon by Portal and Collinge following severe losses a few years ago.

Of recent years, 1927, 1928, the latter part of 1930 and 1931 are all looked upon as being bad years when the losses have been severe and have been recorded, according to Portal and Collinge in their report as "enormous," "exceeding anything that we have previously known" and other similar phrases. The reason for this low frequency is not immediately obvious. The disease in its epidemic form is believed by some game keepers to show a periodicity and to run in cycles. More probably, however, it depends on a number of factors not the least important of which are climatic conditions. The years 1933 and 1934 have been exceptionally dry. As the larva of *T. tenuis* depends at one stage of its life-history upon the presence of water, this drought must undoubtedly have brought about the death of hundreds of infective larvae and therefore have materially reduced the incidence of the disease. Further it has been noticed during the past months that heavily infested birds have come in more frequently about a month or so after a short wet spell.

From these 205 birds carrying helminths, 15 species of parasites have been obtained. No new species will be described but there are 7 new records.

Immediately following is given the number of occasions each parasite was obtained :—*Trichostrongylus tenuis*—207 : *Syngamus trachea*—29 : *Capillaria longicollis*—44 : *C. retusa*—7 : *Heterakis gallinae*—55 : *H. sp.*—1 (larval forms only were present but those in large numbers in the wall of the caeca) : *Davainea proglottina*—3 : *Hymenolepis carioca*—4 : *H. anatina*—1 : *H. linea*—1 : *H. phasianina*—11 : *Choanotaenia infundibulum*—11 : *Raillietina polyuterina*—1 : *R. echinobothrida*—4 : *R. tetragona*—3.

Of these *C. retusa*, *D. proglottina*, *R. tetragona*, *R. echinobothrida*, *H. carioca*, *H. anatina* and *H. phasianina* are here reported for the first time from the partridge (*Perdix perdix*).

Earlier workers have recorded other helminths as parasites of partridges which have not, however, been found in the present survey. They are *Lemdana marthae*, *Ascaridia compar*, *Raillietina globirostris*, *R.* (*Skrjabinia*) *circumvallata*, *Hymenolepis microps* and *Davainea urogalli*.

NEMATODA.

Trichostrongylus tenuis (Mehlis, 1846).

The presence of this parasite in the partridge and other game birds has long been known, particularly in the grouse in which genus it has occasioned serious losses from time to time. The present writer is here following the lead of Cram & Wehr in considering that the form from the partridge—*T. tenuis*—is identical with that from the grouse—*T. pergracilis*. They show that morphologically there is insufficient evidence to separate the two species and both forms give rise to similar pathological lesions and disease symptoms. The partridge would seem to be somewhat susceptible to the action of the parasite. Whereas the grouse has been found to harbour several hundreds of these worms, as reported by Shipley & Leslie, without serious symptoms on many occasions, the highest number found in the partridge in this survey has been 1,531. This, further, is a smaller figure than that given by Portal & Collinge in their report, which however is based on material obtained in years when the disease was rampant. But even with these low figures, typical lesions have been noticed in certain birds and the fatal dose has been as low as 500, though there have been caeca apparently healthy which contained more. Only when the lesions were severe and when marked emaciation was present, was disease attributed to this worm. The caeca were distended and the blood vessels showed themselves much congested. The mucosa was considerably inflamed and the longitudinal ridges were greatly thickened, *i.e.*, there was an acute typhlitis. The contents of the caeca under disease conditions were yellowish-white, dry, caked and of a cheesy consistency. The birds showed evidence of malnutrition, being considerably emaciated. Full grown specimens often weighed only

5–6 ounces as compared with the normal weight of 13–14 ounces. These are the recognised symptoms of strongylosis and they were all found in the partridges which are recorded here as dying of this disease. Portal & Collinge state that there is a darkening of the plumage in diseased birds but this was not observed during the course of this survey.

Syngamus trachea (Montagu, 1811).

This gapeworm was the cause of death in 15 birds. They were all young. Though essentially a parasite of young stock, it has been found in January and February when the birds must have been more than 6 months old, as partridges normally hatch in the middle of June. The symptoms are now almost classical and need not be treated again here. Thirteen pairs of gapes was the highest number recorded from a partridge. The usual number was about 3 or 4 though many birds contained a single pair and others 11 or 12.

Capillaria longicollis (Rudolphi, 1893).

This is a common parasite of the small intestine of many gallinaceous birds. Usually it seems to occasion no damage and though in two birds 23 specimens were recovered yet usually there were only 3 or 4.

Capillaria retusa (Raill., 1893).

This parasite of the caeca has previously been recovered from *Gallus gallus* and *Numida meleagris* but this would seem to be the first record of it in the partridge. There were found 7 birds harbouring it. Single specimens only were found except in one bird which had 4.

Heterakis gallinae (Gmelin, 1790).

It is widely found in domesticated birds and has been previously recorded from the partridge. Its numbers in this series of birds were always small, being generally under 20. It seems to do no harm to its host for the caeca showed no lesions that could be attributed to its presence.

Heterakis sp.

It was not possible to identify this form as no adults were present. The host was a nearly adult cock bird weighing 12 ounces from a shoot

in Norfolk. When the viscera were removed, the caecal walls were observed to be thickened. A complete longitudinal incision having been made, small whitish papillae were observed all over the distal three-quarters of the mucosa. The contents were normal in appearance and consistency, and there were no parasites present in the lumen. Sections of the caeca showed the presence of larval nematodes within the mucosa. *Heterakis isolonche* Linstow, 1906, a fairly common parasite of the pheasant, enters the caecal wall during the early stages of its parasitic existence. We may have a case here of its presence in the partridge but as only two other birds were sent in from this shoot and neither contained any Heterakis, it is impossible to assign a species to this parasite. So far *H. isolonche* has not been recorded from the partridge.

CESTODA.

Hymenolepis linea (Goeze, 1782).

This diagnosis is given with some hesitation. The host was a Hungarian hen bird which had died immediately on its arrival in England but which was not received at the Institute of Agricultural Parasitology for some days and hence was not well preserved. The species was described originally by Goeze from the partridge and has since been recorded from the lark, quail, thrush and "Anser," a series of unrelated birds. There seems, however, to be considerable variation in the accounts recorded by various workers. Krabbe identifies as *H. linea*, a cestode which reached a length of 10 mm. and a breadth of 0·5 mm. and yet was not mature. Wolffhügel on the other hand had a mature form 5 mm. in length and 0·4 mm. broad. He found them in large numbers. The present material consisted of a single worm 4·5 mm. long: it was not mature. The scolex carried 8 hooks 0·22 mm. long. There were 3 testes lying so as to form a right-angled triangle, *i.e.*, the two aporal testes were lying directly in front of one another in the youngest segments, though they became somewhat displaced as they matured. The genital pores were unilateral. The diagnosis was made on the number and size of the hooks, though in the original description Goeze states that the proboscis is apparently unarmed " . . . das Rüsselchen scheinbar unbewaffnet." *H. linea* is the only species of Hymenolepis that has so far been recorded from the partridge. There now follow three more.

Hymenolepis phasianina (Fuhrmann, 1907).

H. phasianina has been recorded already from the pheasant in Europe and Egypt. It has been found 11 times in partridges in the course of these observations and it has rarely been solitary. Specimens usually numbered 5 or 6. It would seem to be fairly prevalent in England as this material was obtained from as widely separated areas as Norfolk, Cambs., Berks., Hants. and Yorkshire.

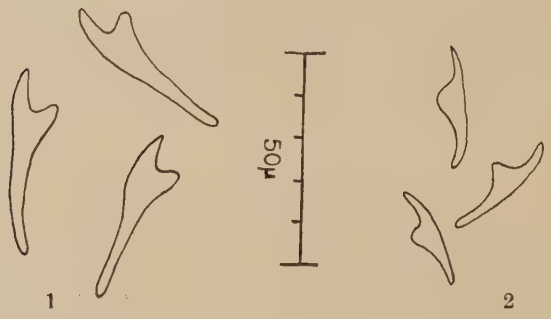


Fig. 1. *Hymenolepis phasianina.* Fig. 2. *H. linea.*

Hymenolepis carioca (Magalhaes, 1898).

H. carioca has been recovered from the fowl in Europe, Asia and America. It occurred 4 times in this group of partridges: as a general rule solitary specimens were found, though one bird had 5.

Hymenolepis anatina (Krabbe, 1869).

H. anatina has previously been obtained from a variety of hosts in Europe. A single worm was recovered from a cock bird from Hampshire. The specimen was rather small being only 8 cm. by 2·5 mm.: it was, however, not mature. The method of fixation may have caused a shrinkage furthermore.

Choanotaenia infundibulum (Bloch, 1779).

C. infundibulum has been already recovered from the partridge. It occurred 11 times in the present survey. The highest number of scolices removed from one bird was 12. The dimensions of the worm are variously given as 5–20 cm. long. In these cases the range was 6–13 cm. The intermediate host is *Musca domestica*, of which the partridge is very fond.

Davainea proglottina (Davaine, 1860).

This small tapeworm, which consists normally of only 4–9 proglottides, occurs in the duodenal loop of the fowl in most parts of the world and has been recorded from the ruffled grouse in America. In the present material it occurred 3 times. From two birds were recovered 132 and 156 scolices respectively but the third bird contained many hundreds. The death of this bird was due to these worms. There was a severe enteritis and considerable haemorrhage caused by the burrowing activities of the cestodes into the duodenal wall. The parasite has not been previously recorded from the partridge.

Raillietina echinobothrida (Mégnin, 1880).

This cestode, which has previously been recorded from the chicken, pigeon, and pheasant, was found in 4 partridges from various parts of England. One bird contained 5 young worms, another contained 3 and two others each contained a single worm. This is a new record for the partridge.

R. (Raillietina) tetragona (Molin, 1858).

R. (*Raillietina*) *tetragona*, a cosmopolitan parasite of the chicken, has not yet been recorded from the partridge. It was found three times and 6 specimens were in all recovered.

R. (Skrjabinia) polyuterina (Fuhrmann, 1909).

A heavy infestation with this cestode occurred in a single bird. It has been recorded from this host before.

Fourteen species of helminths, 6 of these being nematodes and the others cestodes, together with one unidentified species of Heterakis, have been recovered from English partridges. No trematodes or Acanthocephala have been found. Of the new records 6 are of cestodes and the other is a nematode. The 2 species of Raillietina are of cosmopolitan distribution and the fact that they occurred in a number of birds from different localities in England, seems to show that the infestation is not accidental but that *Perdix perdix* should be included as a normal host of these species. The Hymenolepis species are also of fairly wide distribution and the frequency of *H. phasianina* shows that it is probably widespread in this country.

If the life-histories of these cestodes are considered, *R. echinobothrida*, *R. tetragona* and *C. infundibulum* all have Musca as intermediate host and it is known that the partridge catches large numbers of flies for food, especially for the young. Similarly with *H. carioca*, the intermediate host is another fly—Stomoxys. The two species of Raillietina according to some authors have a species of Helix as intermediate host and *D. proglottina* certainly has another mollusc—Limax—as vector. This suggests that the bird may feed on snails also. It is not yet known if partridges actually seek out water for drinking purposes though young birds are observed to drink when water is supplied, when they are reared in captivity. The presence of *H. anatina* which has some of the Cyprinidae, fresh water copepods, as intermediate hosts, suggests that they do drink normally in the wild state. The usual definitive host of this cestode is a duck.

Parasites of Other Birds.

Occasionally other birds came in for examination and these are recorded as certain findings are interesting, though the numbers remained small.

Six specimens of the Red Leg Partridge (*Alectoris rufa*) were examined. It is generally believed that this species is hardier than the British partridge, *Perdix perdix*. Portal & Collinge say that " . . . in some years when the English one (*i.e.*, partridge) had fallen to a very low figure, the Red Leg remained on a high one, and in some average years the Red Leg exceeded the other." And later " . . . its apparent greater stamina and powers of resistance may be due to the fact that its introduction into England is comparatively of recent date, namely about 1770.

Or also to the fact that the Red Leg (*Caccabis rufa*, L.), not being a true partridge may be immune to these forms of disease." (*Caccabis rufa* (L.) = *Alectoris rufa*).

Six birds are not enough to generalize from but it is interesting to note that 2 of them contained *T. tenuis* in appreciable numbers—one had 328 and the other 457. This shows that the Red Leg is a possible host for this parasite and the likelihood of disease cropping up should not be overlooked. Three birds contained *S. trachea* in sufficiently large numbers to cause death, 2 contained a few *H. gallinae* and 1 bird contained 5 scolices of *H. phasianina*. It would seem that the helmintic fauna of *Alectoris rufa* is worth looking into now that it is being so freely bred in England for shooting purposes.

Twelve pheasants were also examined, 6 of which had succumbed to gapeworm infections—one bird indeed carried 30 pairs of worms in the trachea. Other parasites recovered were *H. gallinae*, in one case there being 1,210 specimens, and *C. longicollis*. All these forms have been recorded from *Phasianus colchicus* before.

From a quail, *Coturnix coturnix*, were recovered 7 scolices of *Metroliasthes lucida* (Ransom, 1900) which has not yet been recorded as a parasite of this host but only from the turkey and hen, while the grouse, *Lagopus scoticus* furnished only the usual *Davainea urogalli*, *Hymenolepis microps* and *T. tenuis*.

Summary.

1. Examinations for helminths have been made on 380 English partridges (*Perdix perdix*), 6 Red Leg partridges (*Alectoris rufa*), 12 pheasants (*Phasianus colchicus*), 3 quails (*Coturnix coturnix*) and 7 grouse (*Lagopus scoticus*).

2. From *Perdix perdix* 6 species of nematodes and 9 cestodes were recovered. Seven of these, *C. retusa*, *D. proglottina*, *H. carioca*, *H. anatina*, *H. phasianina*, *R. echinobothrida* and *R. tetragona* are recorded for the first time from this host.

3. From *Alectoris rufa* were obtained 4 species of helminths, none of which had been recorded from it before. They are *T. tenuis*, *S. trachea*, *H. gallinae* and *H. phasianina*.

4. From *Phasianus colchicus* 3 species were obtained. They were all nematodes. From *Lagopus scoticus*, 2 species of cestodes and one nematode, all of which had been found in these hosts before.

5. A new cestode infestation, *Metroliasthes lucida*, was noted in the quail (*Coturnix coturnix*).

References.

Cram, E. B. & Wehr, E. E., 1934.—" The Status of species of Trichostrongylus in Birds." *Parasitology*, xxvi (3), 335-339. (*W.L.* 16035.)

Fuhrmann, O., 1907. " Bekannte und neue Arten und Genera von Vogeltänien." *Zbl. Bakt.*, xlv (6), 516-536. (*W.L.* 23683.)

Goeze, J. A. G., 1782. " Versuch einer Naturgeschichte der Einegeweidewürmer thierischer Körper." Blankenburg.

Krabbe, H., 1869. " Bidrag til Kundskab om Fuglenes Baenelorme." Pub. Bianco Lunos Bogtrykkeri ved F. S. Muhle. Kjøbenhavn.

Leslie, A. S. & Shipley, A. E., 1911.—" The Grouse in Health and Disease." The Report of the Committee of Inquiry on Grouse Disease. Pub. Smith, Elder & Co.

Lühe, M., 1910. " Parasitische Plattwürmer. 11. Cestodes. Heft 18 of Die Süsswasserfauna Deutschlands." edit. Brauer Pub. Gustav Fischer, Jena.

Portal, M. & Collinge, W. E., 1932.—" Partridge Disease and its Causes." Pub. Country Life Ltd.

Sprehn, C. E. W., 1932.—" Lehrbuch der Helminthologie." Pub. Gebrüder Borntraeger.

Wolffhügel, K., 1900.—" Beitrag zur Kenntnis der Vogelhelminthen." Inaug. Dissert. der Univ. Basel. Pub. Univ. Freiburg. i. Br.

Journal of Helminthology, Vol. XIII, *No.* 3, *July*, 1935, *pp.* 149–158.

On the origin of strains of *Heterodera schachtii* occurring in Britain, with special reference to the Beet-strain.

By Marjorie J. Triffitt, D.Sc.

(*Field Officer, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

The history of the beet-strain of *Heterodera schachtii* in Germany, where it was first recognized in 1859, and its subsequent discovery in other beet-growing European countries and the United States of America, have already been described in some detail in a Memorandum published by the Imperial Bureau of Agricultural Parasitology. This Memorandum was published in 1931 with the object of emphasizing the potential danger of *Heterodera schachtii* to the sugar-beet industry, then comparatively newly established in Britain. Observations carried out in America are quoted, which strongly indicated that the eelworm was introduced into the United States by the use of seed imported from European countries where the infection was known to be intense and of long standing ; this contention, however, does not yet appear to have been decisively proved. The danger of repeated beet cultivation, without regard to rotation of crops, was particularly emphasized with reference to the wide distribution of the potato-strain of *H. schachtii* in Britain, and such infections as were already known to occur on other host-plants were noted.

In the autumn of 1934 it was discovered by Petherbridge that the beet-strain of *H. schachtii* was already present and sufficiently well established to cause damage to crops in the Isle of Ely. Subsequent records show that infected beet has been received at the factories at Ely, Lyn, Bury St. Edmunds, Peterborough and Selby, and some of these infections have been traced to the Isle of Ely, W. Norfolk, W. Suffolk and Cambridgeshire. A previous record of the existence of a strain of *H. schachtii* in Gloucestershire which formed a natural field infection on mangolds, Brassica and certain weeds including three species of grasses and one of *Rumex*, was made by Walton and Staniland (1928). That this strain also attacked sugar-beet grown experimentally in infected soil was shown by Triffitt (1929). In 1932 Thompson recorded a partial

failure on mangolds at Llanischen (Glamorgan) associated with *H. schachtii*, and stated that mangolds on adjoining ground had failed similarly the previous year.

From these records it appears that the beet-strain of *H. schachtii* is now widely distributed in Britain although it is less well known than the strains attacking potatoes and peas. It is a generally accepted fact, particularly in the case of the beet-strain, that, except where the host-plant is grown repeatedly without regard to rotational cropping, a slight initial infection in the soil tends to multiply and spread comparatively slowly. It is equally well recognised that until damage to the crop becomes evident the presence of eelworm normally goes unsuspected.

That repeated cropping of the same land with sugar-beet has been practised by certain growers is shown by the records of the Agricultural Advisory Officers. The first case of " beet sickness " reported by Petherbridge occurred on land which had grown beet or mangolds ten times in the past fourteen years, and Thompson records cases where beet has been grown for 9, 6 and 5 years in succession on land heavily infected with the potato-strain of *H. schachtii*. It is of special interest that none of the cases reported and investigated by Thompson showed any evidence of the potato-strain having produced an infection on beet.

As opposed to the growers who have cultivated beet continuously, it is probable that the majority of farmers have adopted beet as a fallow crop in a three or four course rotation; this more especially so since a warning against the dangers of repeated beet cultivation was issued in leaflet form by the Ministry of Agriculture and reprinted by certain periodicals. Hence it would seem highly probable that small foci of infection may have been established, which, under a rotational system of cropping have not caused noticeable damage to the crops. In support of this suggestion a series of observations by the present writer may be quoted. During the examination of soil samples undertaken in connection with research work on the potato-strain of *H. schachtii*, lemon-shaped cysts of the type associated with infections on hosts other than the potato, have frequently been found. Several small foci of infections of this type were found in one field which was thoroughly sampled and was known to have grown cereals but not beet or mangolds in the past four years. On examination it was found that only a small percentage of these cysts contained viable eggs and none of the cysts appeared to have been recently

formed. Examinations of weeds gave a negative result and it was not possible to determine on what host plant the cysts had been produced. The absence of newly formed cysts immediately after the cultivation of oats strongly suggested that a cereal-strain was not involved, although a single white stage female was found on the roots of wheat in an adjacent field. Peas had never been cultivated on this land. On another occasion when a composite sample was examined from each of thirty four fields comprising the arable land of four farms, eleven of the samples were found to contain lemon-shaped cysts. Crop records of these farms showed that only two of the eleven fields had grown " roots " during the past five years. As turnips were listed separately it seems safe to assume that " roots " indicated beet or mangolds. In the season succeeding that in which the samples were taken, beet growing in one of the fields which contained lemon-shaped cysts was found by another investigator to bear a slight infection of eelworm.

Early investigations into the method by which the beet strain of *H. schachtii* might possibly have been introduced to the United States of America are described by Shaw. It was found that a certain amount of soil was invariably included in the sacks of imported beet seed. This soil was either in the form of dust adherent to the rough surface of the seed or in small lumps varying up to the size of a walnut. Although cysts had not, up to the date of publication (1915) been found in the soil (the flotation method of extracting cysts from soil was then unknown), this was regarded as strong presumptive evidence that the beet-strain had been introduced to the United States through the agency of imported seed.

Since the majority of the beet seed distributed to growers by the beet factories in Britain is of foreign origin, it has been suggested by the Imperial Bureau (1931) that some measures should be taken to ensure that beet eelworm was not introduced into Britain in a similar manner. Recently a small sample of beet seed imported from Germany has been obtained by the Institute of Agricultural Parasitology. This sample was obtained by the courtesy of a farmer and beet grower who received it from a beet factory in Britain. The seed was sieved and a large quantity of debris, comprising fragments of beet seed and soil dust, was obtained.

It was not possible to separate any cysts that might have been contained in this debris by the flotation method owing to the amount of seed coat fragments present. In addition to the dust, the seed sample was also

found to contain soil in the form of small lumps, varying in size from about 1 mm. diameter to the size of a pea. These were picked out from the seed and found to weigh 3 grammes. They were then crushed and the resultant dust was subjected to the cyst flotation method. One cyst of the beet-strain type was isolated and was found to contain a mass of empty egg membranes and a single embryonated egg. This seems to be conclusive proof of one origin of the beet strain in Britain.

Since imported mangold seed is also used in considerable quantity it seems probable that the mangold-strain in western England, which is probably biologically identical with the beet-strain, was introduced in the same manner; further it appears not impossible that foci of potential infection were already present in the country, owing to the cultivation of mangolds from imported seed, before sugar-beet growing became an established agricultural practice.

Since the discovery that the beet-strain of *H. schachtii* is in fact carried by imported beet seed, further reference must be made to the possibility of treating such imported seed in some way to ensure that the contents of any cysts that are included with it are killed. In the 1931 publication of the Imperial Bureau of Agricultural Parasitology, the results of dry heat treatment of beet seed carried out in America and reported by Shaw, are quoted together with Fuchs's observations on the effects of dry heat on the cysts of *H. schachtii* which also are summarized and discussed in Shaw's publication. Fuchs found that exposure of the brown cysts to a temperature of 63°C. for only one minute was sufficient to kill the cyst contents, while the American workers showed that beet seed was not injured by an exposure of ten minutes to a dry heat of 75°C., and hence recommended a treatment of five to ten minutes dry heat at a temperature ranging between 65° and 70°C.

Recently work has been carried out with the object of checking these results. Beet and mangold seed was subjected to dry heat in a water-jacketed oven at temperatures ranging from 60°C. to 70°C. for periods of from one to ten minutes. The treated seed, together with untreated samples as controls, were sent to the National Seed Testing Station, Cambridge, for germination tests. The writer wishes to express her thanks to the Director of the Seed Testing Station who kindly arranged for the carrying out of these tests.

It was shown that the germinating capacity of the heat treated seed was in no way reduced, in fact the germination appeared to be very slightly stimulated by the treatment, a result which confirms in every respect the findings of the American workers.

Simultaneously with the seed, cysts of *H. schachtii* were placed in the oven in small uncovered petri-dishes, and these were removed, together with the seed at the close of each seed treatment. As it was thought desirable to use a considerable quantity of cysts for each test, potato-strain cysts, which were more readily available than beet-strain cysts, were used. Moreover certain standardized stimulative substances to which the potato-strain was known to react were also available, and it was thought that the most reliable results would be obtained by their use.

On removal from the oven a little distilled water was added to each petri-dish in order that the cysts might become thoroughly soaked. As a control untreated cysts were soaked in the same way. After the lapse of a week it was proposed to add to the water a stimulative substance, which would cause the larvae to hatch if they remained alive. It was found, however, that before any stimulation was applied larvae were already hatching in considerable numbers from every batch of heat treated cysts although no hatching was taking place in the control. The rate of hatching was accelerated by the addition of a stimulative substance, and following its application larvae also hatched freely from the control cysts.

Following these results further batches of cysts were similarly treated for periods ranging from five minutes to one hour at temperatures of 75°C., 80°C. and 90°C. In every case larvae hatched more readily from the heat-treated cysts than from the controls. A sample of beet seed was kept for one hour in the oven at 80°C. and was then planted in clean soil. Rather contrary to expectation some of the seed germinated, but as this germination test was not carried out by the National Seed Testing Station under standard conditions comparative figures are not available. It appears, however, from a comparison of the amount of seed planted with the number of seedlings that only about 60 per cent. germination has occurred.

As it seemed obvious that the survival of the larvae contained in cysts subjected to a dry heat of 90°C. for an hour could only be due to the heat resistant, or non-conductive qualities of the cyst wall, a few cysts were cut

open before being placed in the oven at 90°C. for 15 minutes. No hatching took place from these cysts even when the optimal stimulation was applied.

From these results it appears that the application of dry heat cannot be used as a means of sterilising beet seed against the possibility of its carrying living cysts of the beet-strain of *H. schachtii*. The use of wet heat and chemical treatment is now under investigation and will be made the subject of a separate publication. As a recent publication has tended to imply that hot water treatment of the type used for killing *Anguillulina dipsaci* in bulbs may also be successfully used to kill *H. schachtii* cysts attached to potato " seed " tubers, it may be well to state here that hot water treatment for one hour at 110°F. does not kill the larvae contained in cysts.

Apart from the possibility of introducing infective material of the beet-strain into Britain by means of foreign-grown beet and mangold seed the potential dangers of strains of *H. schachtii* already established in Britain on other hosts were pointed out in the Imperial Bureau's publication. In this connection the inadvisability of repeated cropping of land with beet was particularly stressed.

Since 1931 numerous isolated observations bearing on this aspect of the problem have been recorded. It is thought that these observations and records, when brought together and correlated, may be significant and suggestive of future lines of research on the problem of biological strains which forms a fundamental aspect of many approaches to the control of *Heterodera schachtii*, and on which very little information is at present available.

As early as 1931 the potato-strain of *H. schachtii* was known to be so wide-spread in England and Wales as to be considered almost general in distribution. Infections on other hosts of agricultural importance, such as hops, peas and oats, were known to have occurred sporadically, an infection on sea marram grass had been recorded from the coasts of Devon and Aberdeenshire, while *Chenopodium album*, beet and couch grass growing in land infected with the potato-strain had also been found to bear cysts of *H. schachtii*.

The possibility that any of these strains of the nematode might, under exceptional circumstances, undergo a physiological re-adaptation which would in time enable it to form an intense infection on beet was discussed.

In the summer of 1931 heavy infestations of peas, associated with crop failure, were recorded from several districts almost simultaneously, and the pea-strain of *H. schachtii* has subsequently been found to be comparatively widely distributed both in England and Wales.

Even more recently a strain attacking cereals has been diagnosed in Shropshire (Edwards), Lancashire (Miles), and Hampshire (Hodson), while another natural infection on grasses has been recorded from Yorkshire (Thompson).

Although the potato-strain of *H. schachtii* is known to be very highly specialised and limited in host range, there is definite evidence that it can effect a transference to tomatoes. Morgan found a few cysts on the roots of *Chenopodium album* growing in a field infected by the potato-strain. On two occasions beet grown in land infected by the potato-strain has been recorded as bearing a slight infection of the eelworm. In the first case the infected beet was growing between the rows of potatoes, and in the second a light infestation of beet grown for the second successive year in the same land was recorded. In Germany Goffart has also described a transference from potato to beet. In view of the findings recorded above this evidence appears to be inconclusive, for the possibility of a slight infection of a strain capable of attacking beet being present in the soil together with the potato-strain, or of a few cysts being introduced with the beet seed are not excluded.

One apparent instance of a transference of the potato-strain to carrot is recorded by Triffitt. In this case potatoes had failed so completely as to be ploughed up early in the season, and carrots and parsnips had been sown immediately afterwards. A number of white stage females were found on the roots of the carrots in the early autumn. That this represented an infection of *H. schachtii* and not an infection of *H. marioni* (which was also present in the soil) as suggested by Goffart, was shown by a subsequent morphological study of the brown cysts and their contents which completed their development on material removed to the laboratory. A sack of soil was obtained from this plot and used for the cultivation of carrots in the following year but no infection was found on the roots of the carrots. No lemon-shaped cysts, such as were produced on the carrots were found in the soil, which may therefore have been taken from a patch where no infection existed on the plants. Two possibilities arise with regard to this infection ; firstly the existence of two eelworm

strains in the soil is not excluded, and secondly it seems possible that a small proportion of the potato-strain larvae which were already free in the soil at the time when the carrots were sown, *i.e.*, immediately after the potatoes were ploughed up, may have succeeded in penetrating the roots of an unusual, and possibly unfavourable, host, and completing their life cycle. There is possibly some support for the second suggestion in the apparent transference of the pea-strain to cabbage in somewhat similar circumstances.

The normal host-range of the pea strain has been found by Walton, Ogilvie and Mulligan, to be confined to certain species of leguminous plants, viz., peas, broad beans, vetch and red-clover, but the same observers have found infections on spring-cabbage in many localities of the Evesham district when cabbage has been grown on land infested with the pea-strain of *H. schachtii* immediately following the harvesting of the pea crop. Moreover, the white stage females have been visible on the roots as early as March and April. This suggests that the larvae may have entered the roots in the autumn and completed their development in the early spring. The absence of other infections on cabbage in the district discounts any suggestion of slight unrecognised infections of another strain which includes cabbage in its host range being responsible for these cases.

More recently Edwards has found that the pea-strain is capable of reproducing, under experimental conditions, on radish, mustard and possibly potato.

The discovery of cysts on the roots of couch grass (*Agropyrum repens*) growing in land heavily infested with the potato-strain was recorded by the present writer. An attempt to transmit the infection from couch grass to potato has, however, failed. Carroll has similarly failed to transmit infections from dock to potato and from potato to dock although both plants were found to be heavily infested in the field.

The presence of a natural infection of a species of *Heterodera*, probably *H. schachtii*, has been discussed as a possible origin of infection on agricultural crops. In March 1935 Thompson found a very severe infestation of two grasses, *Lolium perenne* and *Agrostis canina*, on bowling greens in Leeds, and, as already stated, cereals have been found to be severely affected by *H. schachtii* in several districts of England. Edwards has shown that red-clover and both perennial and Italian rye-grass may be attacked by this cereal strain.

Goffart has found that in Germany larvae of the oat-strain will penetrate the roots of *Thalaspi arvense*, *Erysimum cheirantoides*, *Stellaria media* and *Anagallis arvensis*, although the life cycle is not completed on these plants. He points out that of the first three of these plants two belong to the Cruciferae, included in the host group of the beet-strain and one is a common host of the beet-strain. He also found that cysts were produced on the roots of red-clover and that fourteen species of grasses were attacked. Rather curiously, eight species of grasses that appeared to be immune included perennial and Italian rye-grass.

A critical consideration of these host records indicates that some degree of relationship may exist between certain strains of the nematode, and that a possible connection may exist between natural infections on weeds and infections on certain agricultural crops.

Thus it appears that red-clover may be attacked by both pea- and oat-strains ; radish and cabbage by pea and mangold (or beet) strains. The potato-strain may, possibly, attack beet, and the pea-strain may, possibly, attack potato.

The infections on grasses are of special interest in that they link up natural infections of wild plants with infections of cultivated hosts. More particularly is this of importance with regard to the cereal strain of the nematode, for, although the beet-strain may be distributed by means of contaminated beet seed, and the potato-strain is known to be carried by cysts adhering to the " seed " tubers, the mode of distribution of infections of cereal and pea strains remains unknown.

Natural infections on sea-marram grass may possibly be connected with the occurrence of infections on *Lolium perenne* and *Agrostis canina* on bowling greens, since the turf used for such purposes is commonly obtained from coastal areas. Couch grass (*Agropyrum repens*), annual meadow grass (*Poa annua*) and perennial rye-grass (*Lolium perenne*) have been found to bear cysts when growing as weeds on land infected with a strain attacking mangolds, which is probably identical with the beet-strain, and both perennial and Italian rye-grass were found to be strongly infested by the oat-strain in an experimental series of transmission tests. Further the possibility that the Ormskirk infection on couch grass was derived from the potato-strain is not entirely ruled out, while the pea-strain can attack three alternative hosts of the beet-strain, viz., cabbage, radish and mustard, other alternative hosts of the beet-strain being certain

grasses. Finally, one of the first cases of *Heterodera* attack on wheat described in this country was produced by Theobald, who grew wheat in soil mixed with the roots of infected hop plants.

Although there is no definite evidence that *H. schachtii* infections naturally occurring on grasses have been responsible for the origin of any strain of the eelworm attacking a host of agricultural importance, the possibilities implied in the scattered records and observations which have been brought together and summarised above may be of significance.

References.

Carroll, J., 1933.—" A Study of the Potato Eelworm (*Heterodera schachtii*) in the Irish Free State." *J. Helm.*, Vol. xi, No. 3, pp. 137-156. (*W.L.* 11224b.)

Edwards, E. E., 1935.—" On *Heterodera schachtii*, with special reference to the Oat race in Britain." *J. Helm.*, Vol. xiii, No. 2, pp. 129-138. (*W.L.* 11224b.)

Fuchs, O., 1911.—" Beitrage zur Biologie der Rübennematoden *Heterodera schachtii*." *Z. Landw. Vchsw. Öst.*, Jahrg. 14. pp. 923-949. (*W.L.* 23488.)

Goffart, H., 1928.—" Verwandtschaftliche Beziehungen zwischen dem Rüben- und Kartoffelstamm von *Heterodera schachtii* Schm." *Verh. deuts. Zool. Ges.*, 32 Jahresversammlung, 1928, pp. 238-243. (*W.L.* 22270.)

——, 1932.—" Untersuchungen am Hafernematoden *Heterodera schachtii* Schm. III. Beitrag zu Rassenstudien an *Heterodera schachtii* Schm." *Arb. biol. Reichsanst f. Land- u. Forstwirts.* Bd. xx, Heft 2, 26 pp.

——, 1934.—" Beobachtungen au *Heterodera marioni* (Cornu 1879) Goodey 1932 unter besonderer Berücksichtigung ihres parasitologischen Verhaltens." *Z. Parasitenk.* Bd. vii, Heft 1. pp. 61–70. (*W.L.* 32536a.)

Morgan, D. O., 1925.—" Investigations on Eelworm in Potatoes in South Lincolnshire." *J. Helm.*, Vol. iii, No. 5, pp. 185-192. (*W.L.* 11224b.)

[Petherbridge, F. R.], 1934.—" Beet Sickness present in England." *J. Minist. Agric.*, Vol. xli, No. 9, pp. 825-827. (*W.L.* 11365.)

Shaw, H. B., 1915.—" The sugar beet nematode and its control." *Sugar*, Chicago, Vol. xvii (2-9), Reprint 55 pp. (*W.L.* 20721.)

Theobald, F. V., 1909.—" Wheat attacked by *Heterodera*." J. S.-E. Agric. Coll., Wye, Vol. xviii, p. 142. (*W.L.* 11557.)

Triffitt, M. J., 1929.—" Further Observations on the Morphology of *Heterodera schachtii*, with Remarks on the Bionomics of a strain attacking Mangolds in Britain." *J. Helm.*, Vol. vii, No. 3, pp. 119-140. (*W.L.* 11224b.)

——, 1929.—" On the Occurrence and Significance of *Heterodera schachtii* infesting certain Weeds." *J. Helm.* Vol. vii, No. 3, pp. 215-222. (*W.L.* 11224b.)

——, 1931.—" On the Occurrence of *Heterodera radicicola* associated with *Heterodera schachtii* as a Field Parasite in Britain." *J. Helm.*, Vol. iv, No. 4, pp. 205-208. (*W.L.* 11224b.).

——, 1931.—" On the Eelworm *Heterodera schachtii* as a potential danger to the Sugar-beet Industry in Britain." Imp. Bur. of Agric. Parasit. Notes and Memoranda No. 1. 8 pp. [M. MacM.]

Walton, C. L., Ogilvie, L. & Mulligan, B. O., 1933.—" Observations on the pea strain of the eelworm *Heterodera schachtii* and its relation to ' pea sickness,' " Long Ashton (Bristol). Ann. Rep., pp. 74–85.

Journal of Helminthology, Vol. XIII, *No.* 3, *July*, 1935, *pp.* 159–162.

The Vinegar Eelworm in Tan Liquor.

By B. G. Peters, M.Sc., Ph.D.

(*From the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

Three eelworm-infested samples of a liquor used for tanning hides were recently forwarded to Prof. R. T. Leiper by Dr. D. Jordan Lloyd, Director of Research to the British Leather Manufacturers' Research Association. I am indebted to Prof. Leiper who identified the worm as *Turbatrix aceti* for the opportunity of examining and reporting upon this material.

The three samples were in an active state of fermentation, having a copious frothy scum floating on the liquor, which itself was of a dark mahogany colour and had the pungent smell associated with a tannery. A rough colorimetric test showed that the reaction of the liquor was about pH : 5·0.

The eelworms were very numerous. Many of them crawled up the sides of the bottles above the level of the scum, giving the appearance in the mass of a fine white network of slowly waving fronds, such as has been described by Fülleborn in the case of infective hookworm larvae. If some of the scum was poured out into a flat dish, a shimmering effect was produced by the constant movement of countless eelworms at the surface. In a suitable light this was just visible to the naked eye.

A cursory examination of the present material was sufficient to show that the eelworm was *Turbatrix aceti* (Muller, 1783), the vinegar eelworm. The absence of the prismatic pharynx found in *Rhabditis*, the double-bulbed oesophagus, the single gonad in the female (the posterior gonad is represented merely by the " ovarium," a hollow sac which probably functions as a receptaculum seminis), the sigmoid spicules and the arrangement of the caudal papillae in the male, all were characteristic of that species.

Detailed measurements, however, showed that this worm from tan liquor differed noticeably in size and proportions from the worm commonly found in vinegar. De Man (1910) described two forms from

vinegar: a larger "A-population" which is the more usual form, and a "B-population" of worms only about half the size and displaying differences in the body proportions. In the same paper he described another form, which he regarded as a distinct variety, *dryophila*, from the white slime-flux of the oak. The latter was of the same size as the B-population from vinegar but had, in the male, a tail longer than the oesophagus whereas the reverse was true of the B-population.

Turbatrix aceti material	De Man's A-population.	De Man's B-population.	De Man's v. *dryophila*.	Eelworm from tan liquor.
Females—				
Length, mm. ...	1·74–2·43	0·96–1·07	0·96–1·36	1·01–1·31
α ...	43–55	35–40	27–40	35–39
β ...	9·0–10·0	5·4–6·0	6·5–7·1	6·4–8·0
γ ...	6·2–7·6	4·8–5·5	3·4–5·3	6·0–7·2
Males—				
Length, mm. ...	1·50–1·81	0·87–1·00	0·80–1·10	0·80–1·08
α ...	50–60	40–42	29–45	24–40
β ...	7·0–8·5	5·0–5·2	5·4–6·3	5·0–6·8
γ ...	7·2–8·4	6·0	4·4–5·7	6·7–8·3

Table of Dimensions and Ratios.

The eelworms from tan liquor are also of this smaller size, namely about 1 mm. in length. In the males the oesophagus is longer than the tail, suggesting affinity with De Man's B-population. But in both sexes the tail is much shorter relatively to the body-length than in the B-population, and therefore *a fortiori* than in the variety *dryophila*. This will be clear from the accompanying table of lengths and of the ratios: length/mid-diameter (α), length/length of oesophagus (β), and length/length of tail (γ), given for each of the forms mentioned above. It will be seen that, in relative tail-length, the tan eelworm is more like the larger A-population.

In a previous publication (1927, pp. 194–200) I have discussed variation in the vinegar eelworm and have suggested that the nature of the surrounding medium may be an important influence. Attempts are now

being made to culture the tan eelworm in vinegar to see whether the body-ratios are changed, in the course of generations, in a different medium. Meanwhile it is not considered desirable to introduce a new variety name for this form.

The origin of the eelworm in tan liquor is not yet known, but an obvious explanation suggests itself. Oak bark is used in the preparation of many liquors, and it is probable that bark affected by slime-flux has been introduced from time to time. *Turbatrix aceti* var. *dryophila* has been found in the white slime-flux of the oak, and not only by De Man. Through the courtesy of Dr. T. Goodey I am able to report the discovery of this same eelworm, together with *T. ludwigii* (De Man, 1910), in a slime-flux collected from an oak near Brockenhurst by Mr. Basil Goodey in the summer of 1933 (the worms are still living, on sliced apple in a refrigerator). Thus it is known to occur in slime-fluxes in this country and may be far commoner than is supposed.

Leather manufacturers are concerned about blemishes of unknown origin which frequently occur on the surface of leather. Monthly Circular No. 5 of the British Leather Manufacturers' Research Association (May, 1935) refers to nematode worms in great numbers on " run " sheepskin pelts during the sweating process, and states that " The pitted markings found after sweating on the flesh and grain of some sheepskins have been attributed to their boring. . . . it may be that they are capable of secreting a proteolytic enzyme." Since the tanning process occurs subsequently to the sweating process it is not likely that the worm in question is *Turbatrix aceti*. Cobb (1925) has found *Rhabditis icosiensis* on sheep skins and has attributed pitted markings on the skins to the activity of this worm during the sweating process.

The Circular also refers to worms in great numbers in a quebracho tan liquor, but " There was no suggestion in this case of pitted grain and the finding of the worms was more or less accidental." From what is known of the biology of *T. aceti*, it is probable that it feeds upon bacteria or similar microscopic organisms. I am aware of no evidence for the active secretion of proteolytic enzymes by this worm. Hence there is no reason, at present, to suppose that the vinegar eelworm is able to damage hides and skins during the tanning process.

References.

Cobb, N. A., 1925.—" *Rhabditis icosiensis*, injurious in dewooling sheepskins." Helminthological Society of Washington, in *J. Parasit.*, xi, p. 218. (*W.L.* 11428.)

Man, J. G. de, 1910.—" Beiträge zur Kenntnis der in dem weissen Schleimfluss der Eichen lebenden Anguilluliden." *Zool. Jb.*, Abt. 1, xxix, 359-394. (*W.L.* 23831.)

Peters, B. G., 1927.—" On the anatomy of the vinegar eelworm." *J. Helminth.*, v (4), 183-202. (*W.L.* 11224b.)

Journal of Helminthology, Vol. XIII, *No.* 3, *July*, 1935, *pp.* 163–166.

Further Observations on the Incidence of Parasitism of Flea Beetles by the Nematode, *Howardula phyllotretae.*

By J. N. Oldham, B.Sc., Ph.D.

(*Senior Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

During a study of the nematode, *Howardula phyllotretae* Oldham, 1933, parasitic in Flea Beetles of the genus *Phyllotreta*, records were kept in order to get data concerning the incidence of infection. The records already published, with a description of the parasite (Oldham, 1933), refer solely to the observations made during 1931. During the two subsequent years large numbers of beetles belonging to the following species: *P. atra*, *P. cruciferae*, *P. nigripes*, *P. undulata* and *P. vittula*, were collected and examined for parasites. Collections were made, in each year, from about the beginning of April, when the adults started to emerge from their winter shelters, up to the end of September, when the beetles became scarce and were leaving the plants to hibernate. The observations made, therefore, cover the entire period of the insect's active life.

The records for 1931 show an entire absence of parasitism in *P. nemorum*. Adults of British (Hertfordshire) and German (Schleswig-Holstein) origin were studied as well as larvae, extracted from turnip and radish leaves, with negative results. In order to confirm these observations numerous adults of *P. nemorum*, collected during 1932 and 1933, were examined but in no case was parasitism observed. The habit of this beetle, which passes its larval life within leaf tissues and is not a root feeder in the soil, was, and still is, considered by the writer as the only feasible explanation of the lack of parasitism.

The specimens examined in 1932 and 1933 were collected at the Institute's Farm at St. Albans, Hertfordshire, and the following remarks dealing with the relative frequency of the insect species refer to these specimens only. The writer has recorded that *P. vittula* was encountered infrequently in 1931 and not in sufficient numbers to produce reliable data concerning percentage parasitism. In 1932 not a single specimen

was collected and the total catch during 1933 was 24 adults. Some indication as to the relative frequency of occurrence in the remaining four species, *viz.*: *P. atra*, *P. cruciferae*, *P. nigripes* and *P. undulata*, was obtained. Collections of material were made by sweeping rows of turnips and other cruciferous plants with an entomological net. The specimens thus obtained were identified and produced a definite relationship between the numbers of each of the species represented at the time of collection. The summation of the results of these separate collections, carried out over the six months' period of the insects' activity, was made and the resulting figures taken as indicative of the relative frequency with which each species occurred. Thus, in 1932 the ratio which existed between the species, in the order in which they are given above, was 4 : 1 : 1 : 8 while, in 1933, the ratio was 5 : 1 : 1 : 9. *P. cruciferae* and *P. nigripes* were collected in approximately equal numbers in each year while the numbers of *P. atra* and *P. undulata* taken in 1933 were somewhat higher than those for 1932.

An examination of the data obtained shows that, for the years 1932 and 1933, both male and female beetles of all species were parasitized with the exception of *P. cruciferae* during 1933 in which neither sex was found to harbour parasites ; the number of specimens examined, however, was comparatively small. In any case *P. cruciferae* was only lightly parasitized during the two preceding years. Generally there was little difference in the degree of parasitism between the sexes of each beetle species, the incidence being slightly higher in the males practically throughout. The most outstanding feature was the variation in the incidence of parasitism in individual years especially in the case of *P. undulata*. In 1931 the parasitism of the combined sexes of this species was 74 per cent. ; in 1932 it had decreased to approximately 60 per cent. ; while in 1933 it had still further decreased to almost 24 per cent. *P. atra* shows a similar but less well marked decrease in parasitism from 11·36 per cent. in 1931 to 4·67 per cent. in 1933. This state of affairs, however, is reversed in the case of *P. nigripes* where a very slight increase from 10 per cent. in 1931 to 13·33 per cent. in 1933 resulted. If one considers the average incidence of infestation for all species of beetles during each year it will be observed that, in 1931, parasitism was about 26 per cent., in 1932 it increased to practically 38 per cent., while in 1933 it decreased to as low as 16 per cent. No explanation of this fluctuation in percentage

PARASITISM OF *PHYLLOTRETA* SPP. DURING 1931, 1932 AND 1933.

		Number examined			No. parasitized by helminths			Per cent. parasitized by helminths			No. parasitized by insects			Per cent. parasitized by insects		
	Year	♂	♀	Tot.	♂	♀	Tot.	♂	♀	Tot.	♂	♀	Tot.	♂	♀	Tot.
P. atra ...	1931	38	50	88	4	6	10	10·53	12·00	11·36	—	2	2	—	4·00	2·27
	1932	157	250	407	20	22	42	12·74	8·80	10·32	1	—	1	0·64	—	0·25
	1933	254	260	514	11	13	24	4·33	5·00	4·67	8	5	13	3·15	1·92	2·53
	Total	449	560	1009	35	41	76	7·80	7·32	7·53	9	7	16	2·00	1·25	1·59
P. cruciferae	1931	38	48	86	—	2	2	—	4·17	2·33	—	—	—	—	—	—
	1932	52	56	108	6	2	8	11·54	3·57	7·41	—	—	—	—	—	—
	1933	44	41	85	—	—	—	—	—	—	—	—	—	—	—	—
	Total	134	145	279	6	4	10	4·48	2·76	3·58	—	—	—	—	—	—
P. nigripes ...	1931	50	50	100	7	3	10	14·00	6·00	10·00	—	—	—	—	—	—
	1932	37	59	96	6	4	10	16·22	6·78	10·42	1	2	3	2·70	3·39	3·13
	1933	36	54	90	5	7	12	13·89	12·96	13·33	—	—	—	—	—	—
	Total	123	163	286	18	14	32	14·63	8·59	11·19	1	2	3	0·81	1·23	1·05
P. undulata ...	1931	50	50	100	38	36	74	76·00	72·00	74·00	2	—	2	4·00	—	2·00
	1932	380	412	792	243	230	473	63·95	55·83	59·72	1	1	2	0·26	0·24	0·25
	1933	430	480	910	114	103	217	26·51	21·46	23·85	1	3	4	0·23	0·63	0·44
	Total	860	942	1802	395	369	764	45·93	39·17	42·40	4	4	8	0·47	0·42	0·44
P. vittula ...	1933	4	20	24	4	8	12	100·0	40·00	50·00	—	—	—	—	—	—
Total of all species for	1931	176	198	374	49	47	96	27·84	23·74	25·67	2	2	4	1·14	1·01	1·07
	1932	626	777	1403	275	258	533	43·93	33·20	37·99	3	3	6	0·48	0·39	0·43
	1933	768	855	1623	134	131	265	17·45	15·32	16·33	9	8	17	1·17	0·94	1·05
Grand Total		1570	1830	3400	458	436	894	29·17	23·83	26·29	14	13	27	0·89	0·71	0·79

parasitism can be advanced except that it may be linked up with the weather conditions which prevailed during the years in which collections of insects were made. Since *Howardula phyllotretae* presumably has a non-parasitic, free living larval stage in the soil adverse conditions such as a hot dry spring and summer would probably be detrimental to its survival, whereas in a wet season with average or above average temperatures it would find conditions suitable for development to the stage at which it enters its insect host. These assumptions correspond, to some extent, with the results obtained. Thus in 1931 when normal temperatures and rainfalls were recorded for the locality of collection the percentage parasitism was about 26. During the following year while the temperatures recorded were normal or a little above normal the rainfall during late spring and summer was noticeably above the average. Suitable developmental conditions were provided and comparatively little diminution in the nematode population probably occurred. This is reflected in the incidence of parasitism increasing to almost 38 per cent. In direct contrast to the conditions in 1932 the rainfall during the spring and summer of 1933 was considerably below the average and temperatures were consistently high. This hot dry season apparently had a decided inhibitory effect on nematode survival and a marked drop in the incidence of parasitism to 16 per cent. was the result.

While the main object of the writer was to secure information regarding the nematode parasite the occurrence of Hymenopterous parasites was not overlooked and the data obtained during dissections relative to these insects are incorporated in the table. Where parasitism by Hymenoptera occurred the percentage was quite low and of little or no significance.

One phenomenon of these investigations has been the infrequency with which nematodes and Hymenopterous parasites occurred coincidently in a host beetle. Out of the 3,400 insects dissected and examined over the three year period four beetles only, or 0·12 per cent., were found to harbour nematodes and Hymenoptera together. These four specimens consisted of one male and two female *P. undulata* and one female *P. atra* and in each case there was but one Hymenopteron present while the number of adult nematodes varied from one to three.

REFERENCE.

OLDHAM, J. N., 1933.—" On *Howardula phyllotretae*, n. sp., a Nematode Parasite of Flea Beetles (Chrysomelidae ; Coleoptera), with Some Observations on its Incidence." *J. Helminth.*, XI (3), 119-136. (*W.L.* 11224b.)

Journal of Helminthology, Vol. XIII, *No.* 3, *July*, 1935, *pp.* 167–172.

Aphelenchoides hodsoni n. sp., a nematode affecting Narcissus bulbs and leaves.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology. London School of Hygiene and Tropical Medicine.*)

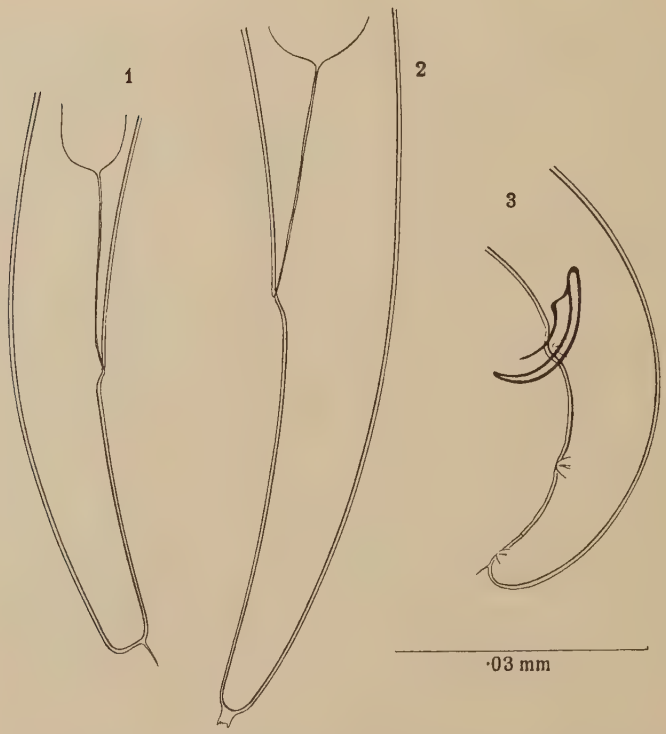
Introduction.

In April 1934 Mr. W. E. H. Hodson, Advisory Entomologist, Reading University, sent the writer a portion of narcissus bulb exhibiting disease symptoms. The bulb was a trumpet daffodil, of the variety "Sir Watkin," from a stock which had been growing in the Isles of Scilly for 40 years or more and had probably never been hot-water treated. It contained a number of nematodes belonging to the genus *Aphelenchoides* which differed in certain anatomical features from other members of this genus. Good specimens of the worm were not very numerous or favourable for detailed investigation but some measurements and drawings were made at the time and it was hoped that at a later date further and better supplies of the organism would become available. In July 1934 the same species of nematode was found in a portion of diseased narcissus bulb sent to the writer by Mr. L. N. Staniland, Advisory Entomologist, Seale-Hayne Agricultural College, Newton Abbot, when the same peculiarities of structure and appearance were again noted and found to be constant.

In April 1935 Mr. Hodson sent a narcissus bulb showing disease symptoms and foliage which was yellow. In both bulb and foliage the worms were again found ; being particularly numerous and large in the yellowed leaves in which, also, there occurred considerable numbers of free eggs and larvae. The same morphological peculiarities were exhibited by these worms as by those found in the bulbs examined in 1934. It was evident that they are constant and constitute certain well defined distinguishing features which separate this from such species as *A. fragariae* and *A. ritzema-bosi*. The new species is named after Mr. Hodson who, with Mr. G. W. Gibson of the Isles of Scilly Experiment Station, has been collecting data on the symptoms of attack and incidence of the disease in the field. It is the intention of these two workers to bring out something on these bionomical aspects at a later date.

MORPHOLOGY.

Dimensions:—*Female*, length, 0·82 mm. to 1·3 mm., α=44–66, β=12·5–19, γ=27·5–32·5, V=65·9% to 73·5%. *Male*, length, 0·75 mm. to 1·01 mm., α=54·5–66, β=11–17·8, γ=23–26, spicules, dorsal pieces, 20μ–21μ, ventral piece, 9μ–11μ, buccal stylet, 11μ–12μ, eggs 48μ–50μ long by 16μ–17μ wide. As the dimensions show, the females may attain



Figs. 1 & 3. *Aphelenchoides hodsoni* n. sp. Female and male tails respectively in lateral view, highly magnified.

Fig. 2. *Aphelenchoides ritzema-bosi*. Female tail in lateral view, drawn to same scale as figs. 1 & 3.

a length well over 1 millimetre and when good numbers of these large forms are found together, as in the affected foliage, they give the impression that a species of much larger size than is usually encountered amongst *Aphelenchoides* species is implicated in this disease of narcissus.

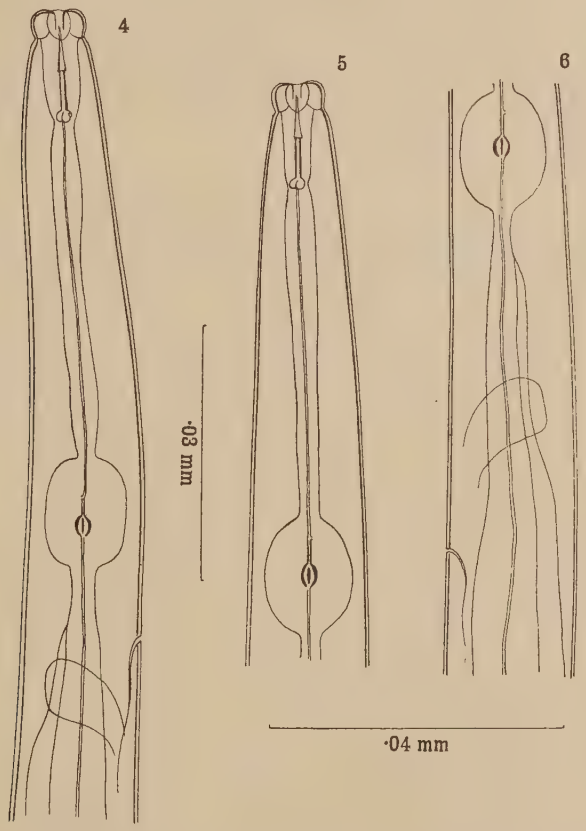


Fig. 4. *Aphelenchoides hodsoni* n. sp. Anterior region of body highly magnified, showing shape of oesophageal bulb, position of crescentic thickenings of lining and location of excretory pore in front of nerve ring.

Figs. 5 & 6. *Aphelenchoides ritzema-bosi*. Corresponding region of body, almost as highly magnified, showing centrally placed crescentic thickenings of lining and the excretory pore behind the nerve ring.

Adults of both sex have a structure typical of *Aphelenchoides*, *i.e.*, they are similar in all essentials of anatomy to *A. parietinus* or *A. fragariae*. The cuticle is transversely striated with fine striae. The head is slightly offset by constriction and exhibits the usual radial ridges dividing it up into six segments. The stylet is typical and bears three basal swellings. There is a muscular oesophageal bulb and the three oesophageal glands lie stretched out in line dorsal to the beginning of the intestine. In the female the gonad is single, has the usual structure and extends anteriorly from the vulva. There is a post-vulval uterine sac. In the male the spicules have the typical thorn shape.

In view of these facts it is scarcely necessary to give a detailed description of the worms but, instead, it is proposed to indicate the chief anatomical features which distinguish them from such a well-known species as *A. ritzema-bosi*, fresh material of which, from diseased chrysanthemum leaves, was available for comparison at the time the new species was being investigated.

1. The female tail is shorter than in *A. ritzema-bosi* and has a different shape, as shown in figs. 1 and 2 which are drawn to the same scale of magnification. In the new species the proportion γ, *i.e.*, length of body to length of tail, is 32·5–27 whereas in *A. ritzema-bosi*, γ is 23–17.

2. The tail in the new species does not taper so much as in *A. ritzema-bosi* and is differently shaped at the end which is blunt, somewhat flattened terminally and carries a small, narrow, pointed spike or process invariably situated towards the ventral side, never centrally. In *A. ritzema-bosi* the tail is shaped as in fig. 2 from which it can be seen that it is not only longer but tapers more and ends in a central terminal process which is not a narrow spike but a kind of boss with an irregularly notched end.

3. The oesophageal bulb is not quite so elliptical in outline as in *A. ritzema-bosi* but has rather flatter sides.

4. The crescentic thickenings of the lining of the oseophageal canal are placed rather far back in the muscular bulb of the oesophagus and are practically always situated behind the centre of the bulb. In *A. ritzema-bosi* they are generally central.

5. The excretory pore is always placed rather far forwards, either at the level of or in advance of the nerve ring, not behind it as in *A. ritzema-bosi*. In this respect the new species resembles *A. olesistus*.

6. The post-vulval uterine sac does not extend quite so far posteriorly as in *A. ritzema-bosi* but generally ends at a point more or less half way between the vulva and the anus.

7. The male tail is typical in shape and in specimens killed by heat it does not take on the characteristic hook bend of the males of *A. ritzema-bosi* but remains rather straight. It carries the same kind of narrow, spike-like terminal process arising, as in the female, on the ventral side. The thorn-shaped spicules and the three typical pairs of lateral caudal papillae call for no special comment.

Discussion.

The foregoing constant anatomical features differentiate the present species from previously described species of *Aphelenchoides* and warrant the creation of a new species : this is accordingly named *Aphelenchoides hodsoni* n. sp.

In view, however, of the fact that this is not the first occasion on which nematodes belonging to the genus *Aphelenchoides* have been reported from diseased narcissus bulbs and foliage, it becomes necessary to justify the creation of a new species.

Cobb (1926) described a nematode under the name *Aphelenchus subtenuis* which occurred in narcissus bulbs and leaves growing in various parts of U.S.A. In the bulbs brown discolouration of the tissues was produced and the leaves were yellowed. The worms were said to resemble *A. ritzema-bosi* very closely and might perhaps be identical with that species. The same author (1928), in a short note presented to the Helminthological Society of Washington, recorded the presence of the same kind of nematodes in diseased narcissus bulbs shipped from the south of France. Unfortunately, neither of these communications by the late Dr. Cobb contained any drawings of the worms. The length of the female is given as 0·9 mm. and of the male as 0·75 mm. but these and other percentage proportions do not carry us far in establishing their identity.

In a short paper by Steiner & Buhrer (1932) dealing with brown-ring symptoms in narcissus bulbs attacked by eelworms in which it is shown that the Stem Eelworm, *Anguillulina dipsaci*, is not the only species giving rise to these symptoms, *Aphelenchoides subtenuis* (Cobb) is made a synonym of *A. fragariae* (Bos) along with *A. olesistus* (Bos) and *A. ritzema-bosi*

(Schwartz). It is apparent from this action by two workers from the same department as Dr. Cobb that they considered there was not sufficient ground for retaining the separate identity of *A. subtenuis*.

If *A. subtenuis* of Cobb is a synonym of *A. fragariae* it cannot be identical with the new species, *A. hodsoni*, the features of which distinguishing it from *A. ritzema-bosi* have already been indicated.

Symptoms.

According to information supplied by Mr. Hodson, in affected bulbs the decayed areas are grey in colour and do not necessarily exhibit ring symptoms though ringing may be present. The leaves turn yellow prematurely ; they may be rather dwarfed and are sometimes split. There is apparently an absence of "spickles" or other leaf swellings.

References.

Cobb, N. A., 1926.—"Nemic Diseases of Narcissus." *The Official Record.* U.S. Dept. of Agric., p. 3.

——, 1928.—"Diseased Narcissus bulbs from France." Proc. Helminth. Soc. Wash., *J. Parasit.*, xv, 68-69. (*W.L.* 11428.)

Steiner, G. & Buhrer, E. M., 1932.—"The Nonspecificity of the Brown-Ring Symptoms in Narcissus attacked by Nematodes." *Phytopathology*, xxii (11), 927-928. (*W.L.* 16273.)

Journal of Helminthology, Vol. XIII, *No.* 3, *July*, 1935, *pp.* 173–190.

Observations on a Nematode Disease of Yams.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

Steiner (1931) published a brief note on the occurrence of disease in a yam tuber, from Jamaica, with which there were associated large numbers of a species of nematode which he placed in the genus *Hoplolaimus*. Steiner and LeHew (1933) published a description of the nematode parasite obtained from this yam and gave it the name *Hoplolaimus bradys*. At a later date Steiner and Buhrer (1934) recorded the same parasite from a yam coming from Porto Rico.

Early in 1934 three small tubes of nematodes from affected yam tubers were sent to the writer for identification from the Imperial Mycological Institute. This material had been received from Mr. J. West of the Department of Agriculture, Ibadan, Nigeria. In July 1934 Mr. West called at this Institute and brought with him some yam tubers which had been grown at Ibadan, in two or three of which the nematode disease was present. Slices made across these tubers revealed the diseased areas just beneath the skin and penetrating a little into the central tissues. The presence of worms was demonstrated in the discoloured tissues and portions of the material were fixed for later examination. Further information was supplied by Mr. West on the incidence of the disease and on the characteristics of its attack. Still later, in the course of correspondence, Mr. West kindly sent particulars of the species of *Dioscorea* which have been found susceptible to attack at Ibadan. The writer desires to express to Mr. West his sincere thanks for the material and information thus generously placed at his disposal, on which it has been possible to conduct the investigation dealt with in the present paper. A brief note on the disease has also been published by West (1934).

Examination of the nematodes revealed the fact that they were closely similar in size and structure to those described by Steiner and LeHew but owing to the fact that lateral caudal papillae were found in adult males and females it became desirable to make a comparison with actual

specimens of the worms studied by these workers. The writer therefore asked Dr. Steiner for the loan of material for this purpose, and in due course he kindly sent two slides of mounted specimens from which it was possible to establish the fact that the species from Nigerian yams is identical with that from Jamaica. Cordial thanks are herewith tendered to Dr. Steiner for the loan of these slides. From the excellently fixed worms originally sent by Mr. West and the diseased yam material obtained later it has been possible to study the structure of the parasite in considerable detail and to make some observations on the pathology of the disease. The results of these investigations are embodied in the following pages.

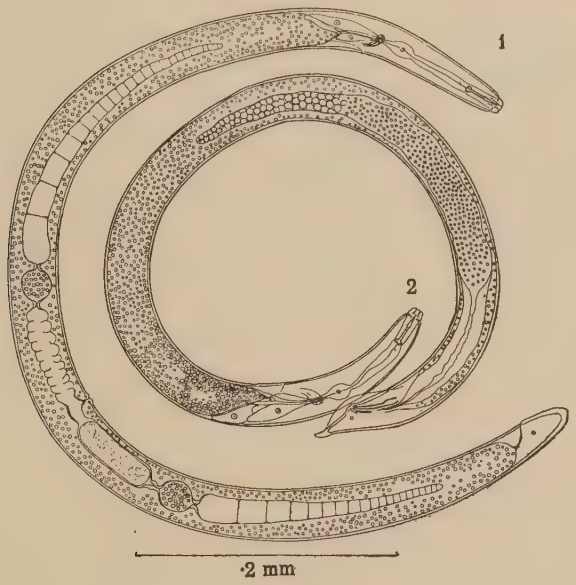
Methods.

The worms sent by Mr. West had been fixed in 5% formalin. They were first washed well in a few changes of water and were then transferred to 70% alcohol containing about 3% of glycerine. The alcohol was allowed to evaporate slowly with the result that the worms were finally left in weak glycerine. By keeping the capsules containing the specimens in an atmosphere dried by means of calcium chloride the glycerine was slowly concentrated and the worms were finally mounted in almost pure glycerine. In this manner well cleared specimens were obtained which revealed the structural anatomy of the parasites in great detail.

In order to study the pathology of the disease, small portions of healthy and affected yam, excised from the tubers brought over by Mr. West, were fixed in a formalin-acetic-alcohol mixture made up as follows :— glacial acetic acid, 5 cc., formalin, 5 cc., 70% alcohol, 90 cc. They were left in this mixture until required for the preparation of sections when suitable pieces were cut out and after repeated washing in 70% alcohol were transferred to iso-butyl alcohol. This was changed two or three times before the pieces were taken over into paraffin wax in which medium they were finally embedded. Transverse and longitudinal radial sections 12μ thick were cut by means of a microtome. Some hand sections were also made. The majority of the sections were stained in Delafield's haematoxylin ; others were stained with Safranin and counter-stained with Delafield's haematoxylin.

Morphology.

Dimensions:—*Female*, length, 0·92 mm. to 1·23 mm., $\alpha = 25{\cdot}6 - 33{\cdot}5$, $\beta = 5{\cdot}9 - 7{\cdot}4$, $\gamma = 27{\cdot}6 - 31{\cdot}5$, $V = 50{\cdot}2\% - 57{\cdot}3\%$. *Male*, length, 0·8 mm. to 0·96 mm., $\alpha = 26{\cdot}5 - 33{\cdot}6$, $\beta = 5 - 6{\cdot}5$, $\gamma = 23{\cdot}2 - 28$, spicules, 30μ–33μ, gubernaculum, 12μ–15μ. As the foregoing measurements and proportions show, the worms are comparatively stout in build. The body tapers slightly in the oesophageal region towards the head which is distinctly offset by constriction. There is comparatively

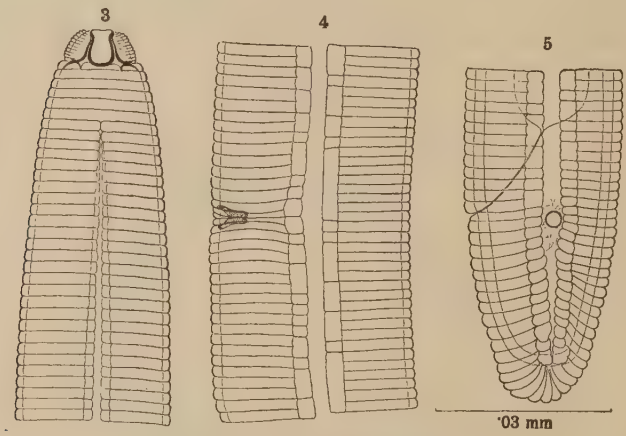


Anguillulina bradys (Steiner & LeHew).
Figs. 1 & 2. Adult female and male respectively to show shape and general anatomy.

little tapering of the body posteriorly and the female tail is broadly conical in outline. The tail end is bluntly rounded and often shows an obtuse flattening on the dorsal side. In cross section it is not completely rounded but is flattened in the lateral fields. The male tail tapers steeply behind the cloacal opening to a conical terminus which is completely

surrounded by the rather voluminous bursa. Both female and male tails are described in greater detail later.

Striations and Lateral Fields.—The cuticle of the head and the body is striated transversely with well defined striae which give the sides of the body a slightly crenate appearance when seen under moderately high magnification. The striae encircle the dorsal and ventral regions of the



Anguillulina bradys (Steiner & LeHew).

Fig. 3. Anterior part of body in surface view, highly magnified, showing arrangement of the cuticular head framework and beginnings of lateral field. Internal organs omitted.

Fig. 4. Surface view of body in vicinity of vulva, highly magnified, showing arrangement of cross markings on outer areas of lateral field in relation to transverse striae of body.

Fig. 5. Female tail in lateral view, highly magnified, showing arrangement of outer areas of lateral field in relation to transverse striae of body. A lateral caudal papilla is shown surrounded by faint markings on inner area of lateral field.

body but are interrupted at the edges of the lateral fields, which extend down each side of the body from a short distance behind the head practically to the end of the tail in the female and on to the bursa in the male. Each lateral field is made up of four lines which, throughout most of the body length, lie about equidistant from one another and thus enclose three longitudinal almost parallel areas. The two outer lines are slightly indented at the points where the transverse striae join them.

In *Hoplolaimus* the outer and inner areas of the lateral fields are said to be striated so as to produce a kind of lattice-like or quadrangular network appearance. At any rate such an appearance has been described by Cobb (1923) as present in *H. coronatus* and by Steiner and LeHew (*l. c.*) in *H. bradys*. For this reason the writer has studied the markings on the lateral fields in 50 or more adults of both sexes obtained from diseased Nigerian yams. The result has been that the markings were found consistently and regularly to follow a common pattern in all cases and it became apparent that no such quadrangular network of lines was constantly present on the lateral fields.

In both male and female worms lying in lateral aspect, when examined under an oil-immersion with a high-power ocular and with good illumination, the lateral fields can be distinguished by careful focusing on the surface of the body. It is then seen that each field begins on about the seventh annulation behind the head. Here a transverse stria, instead of passing right across the body as a plain unbroken band, is interrupted by two flattened convex lines. This may be repeated on the next annulation or two but very soon, on the succeeding rings, these inner convex lines become gradually separated from each other to produce the middle area of a lateral field. The outer lines which mark off the outer areas of a field are very soon apparent as short lines cutting off small portions from each stria as it comes into the lateral region. This arrangement is indicated in fig. 3, where it can be seen that the lateral field starts practically as a single line and then gradually increases in width and has a four line appearance as we proceed posteriorly. In this anterior region of the body, *i.e.*, that part reaching to about the level of the oesophageal bulb, or a little further, practically all the adults examined exhibited regular markings on the outer areas of the field corresponding exactly to the striae. From the inner areas, however, markings of any kind were absent in this region and in the case of only one male specimen did the writer find a few irregular markings on the inner area which did not correspond with the striae and were soon lost. By the time the level of the intestine is reached the regular markings on the outer areas have, as a rule, disappeared and, from this point posteriorly, they become quite irregular in arrangement or disappear entirely until the tail region is reached when they are found again in both sexes. Before describing their appearance on the tail regions a word or two may be given about

the fields in the mid-body region of the female. Fig. 4 is a drawing of a portion of the body surface in the vicinity of the vulva. The outer area on the ventral side is generally deflected a little from the straight line towards the vulval slit. Here the striae lying immediately on either side of the slit are narrower than on the rest of the body. These two, and the one on either side, abut at their inner ends on two larger segments of the outer field. From this point, as can be seen from the drawing, the markings in each direction on the outer fields are quite irregularly spaced in relation to the regular transverse striae whilst in none of the many specimens examined was the writer able to find any markings, regular or irregular, crossing the central area. In very many cases also there are no markings to be seen on either of the outer areas throughout most of the body from the end of the oesophageal region to a point a short distance in front of the level of the anus in the case of the female.

In the latter at about the level where the rectum joins the end of the intestine, markings on the outer areas begin to appear more regularly and soon are found corresponding pretty exactly with the transverse striae as shown in fig. 5. Here and there two striae will be found abutting on a single, somewhat broader, element of the outer areas and at the end of the field there is often an irregular shaped element on either side receiving the inner ends of the striae which now curve round into the longitudinal position. It can be seen that the inner area gradually tapers and disappears at the end of the tail. It is also apparent, on carefully focusing under high magnification, that the inner part of the field is depressed a little below the level of the inner ends of the segmental elements of the outer areas in the tail region.

In the male the lateral fields arise in exactly the same way as in the female and pursue a similar course down each side of the body. There are, as a rule, but few markings on the outer areas and none on the inner area, except in the single case mentioned earlier when some irregular markings were found on a short portion of the neck region. On coming to the beginning of the bursa the inner area gradually widens and the two outer areas diverge ; the ventral one being fairly close to the free edge of the bursa and the dorsal one keeping more nearly to the main longitudinal axis of the body. Each outer area seems to be regularly segmented in correspondence with the striae of the bursa or body. The degree to which these two areas can be traced on the bursa is rather

variable. In most cases it is difficult to trace the ventral area beyond the first 6 or 10 of the bursal striae but occasionally it can be followed further back, as in fig. 8 where it reaches to a short distance anterior to the cloacal aperture. The dorsal area is almost always traceable as far back as the lateral caudal papilla ; its regular segments curving down into the papilla cavity.

Caudal Papillae.—On either side of the female tail, at about the level of the anus, but varying a little in different specimens, there is a lateral caudal papilla which lies in the middle of the inner area of the lateral field. Its outer face is sunk rather below the general level of the surrounding area and here there are often found irregular markings on the surface of the inner area indicating that the cuticle is slightly ridged in producing the shallow hollow in which the papilla lies. Each papilla is apparently almost spherical in shape, has a rather refractive, hyaline appearance and is yellowish in colour. A similar pair of lateral caudal papillae occurs on the male tail and is dealt with later on. Caudal papillae on both male and female were apparently overlooked by Steiner and LeHew.

The rather thick, hyaline body wall is separated from the cuticle at the extreme end of the female body by a fairly wide space so that the cuticle appears almost as though it is being sloughed.

As mentioned in the introduction, Dr. Steiner very kindly loaned the writer two slides of mounted specimens obtained from the Jamaican yam material. One of these slides carried seven adult females and the other a male tail mounted in ventral aspect. The worms had, unfortunately, been fixed in Flemming's solution and were therefore not in a very good condition for high-power observation owing to the blackening of much of the internal organs. All the females showed the lateral caudal papillae and on the male tail the two papillae were also recognized. Of the seven females only three were lying in lateral view and therefore favourable for the detailed examination of the lateral fields. These three, however, each showed markings on the outer areas of the fields, especially in the tail region, exactly similar to those described above. There were faint markings on the inner area just round the lateral caudal papilla and as one proceeded anteriorly the markings on the outer areas became few in number, were irregularly spaced or disappeared, and there were none on the inner area at any rate as far as the vulva. Anterior to the latter

it was practically impossible to follow the lateral field owing to the obscuring effect of the blackened interior of the specimens.

General Anatomy.—The head is distinctly offset by constriction. In outline it is shaped like a truncated cone with slightly convex sides and the anterior face shows a flat central area, about one-third the width of the whole, marked off by a narrow groove on either side. The writer has not observed circum-oral papillae but Steiner & LeHew figure an *en face* view of the head with a minute papilla on each of the four subdorsal or subventral lip regions and an amphid pore on each lateral lip. As already mentioned, the cuticle of the head is transversely striated; the striae being narrower than on the body. The head is supported by a strong cuticularized structure which is yellowish in colour and, when viewed on the surface, has somewhat the appearance shown in fig. 3. From the region of the oral orifice a system of cuticular bands forms loops or festoons, just under the surface of the cuticle, which divide the head into six more or less distinct lip areas.

As one focuses into the depth of the head it can be seen that the cuticular bands form a strong framework which provides a central tubular buccal cavity. The appearances presented by these head structures when seen about half way through the head are shown in fig. 6. The central buccal tube has rounded, knob-like thickenings in front and behind. The outer abutments of the head framework, where the head joins on to the body, have the appearance of a convex piece on either side with points directed backwards. The inner ends appear to be fused to the outer and finer branches of backward extensions of the buccal tube which here forms a spear guide in the median plane. A short distance behind the level of the head, the wall of this spear guide expands outwards as two convex lines, only seen on very carefully focusing and with good illumination. They have very much the same appearance as a similar structure figured by Cobb (*l. c.*) in the corresponding region of *Hoplolaimus coronatus*. The writer has also figured the same sort of lyre-shaped structure in *Anguillulina robusta*, *A. multicincta*, *A. macrura* and *A. obtusa*.

The mouth spear is of stout build and is 26 μ to 30 μ long. It is made up of the two usual portions; an anterior conical and a posterior cylindrical, the former overlapping the latter a little at their junction to form a slight ridge. In specimens undergoing ecdysis it could easily be seen that the anterior conical portion is sloughed along with the head cuticle.

The base of the spear carries three prominent swellings which are rounded posteriorly but on their anterior faces are slightly indented ; the indentations being very difficult to make out even under high magnification and with good illumination. The spear is surrounded by clear bands of muscle which are attached to the head framework and are inserted on the basal swellings of the spear.

The anterior region of the oesophagus is a little wider than the base of the spear and runs posteriorly as far as the median muscular bulb just in front of which it narrows a little. Its canal has a cuticular lining which is continued into that of the bulb where, at the centre, it expands into three small cresentic thickenings. The opening of the dorsal oesophageal gland into the oesophageal canal is a well-marked duct a short distance behind the base of the spear where the canal is deflected out of the longitudinal axis. Behind the median bulb the oesophagus remains rather narrow and forms an isthmus which is crossed by the nerve ring. The oesophageal canal maintains its narrow character and can be traced fairly easily until it joins the beginning of the intestine where it becomes lost. The three oesophageal glands making up the terminal region of the oesophagus are difficult to differentiate at all clearly but seem to arise in front of the narrow beginning of the intestine and just behind the nerve ring where there is a gradual increase in the width of the oesophagus. This expansion is more pronounced on the dorsal than on the ventral side and it seems as though the clear or finely granular tissues composing it wrap round the oesophageal canal and the beginning of the intestine. They are continued posteriorly some distance behind the beginning of the intestine mainly on the dorsal side of the intestine, and in the specimen drawn in fig. 7 the writer satisfied himself of the presence of three lobe-like swellings which probably corresponded to the three gland cells. The three nuclei of these glands are also very difficult to locate with certainty, as they frequently are in species of *Anguillulina*. Two were found in the specimen figured and the third is suggested in dotted outline.

The excretory pore is located at approximately the same level as the beginning of the intestine. As fig. 7 shows, the latter is at first comparatively narrow but gradually increases in width and soon comes to occupy the bulk of the body-cavity. The cells composing it are in most cases crowded with spherical fat globules of a fairly uniform size throughout.

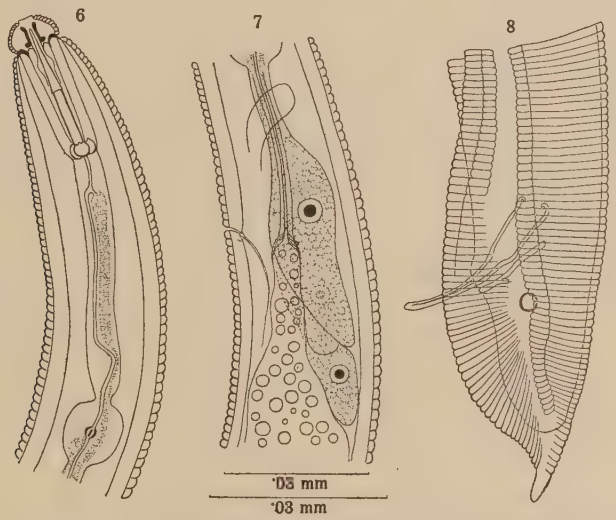
The lumen of the intestine in some specimens can be distinguished as a narrow refractive wavy canal. There is a distinct rectum connecting the end of the intestine with the anus.

Female.—The vulva is not prominent, is sometimes depressed and has only slightly rounded lips. It is slit-like and extends well across the ventral surface of the body. A pair of strengthening thickenings lie at its upper and lower extremities. The gonads are paired, opposed and outstretched ; the end of the anterior ovary coming in some cases to within a short distance of the beginning of the intestine and the end of the posterior one often lying not far from the end of the intestine. The general arrangement of the gonads is shown in fig. 1. Each uterus has a fairly thick wall and is capable of holding one egg at a time. Lying between each uterus and ovary and separated from each by a constriction of the gonad wall is an almost spherical receptaculum seminis which is often found crowded with small round sperms. Its shape and position are very similar to that figured by the writer (1932) for the same organ in *Anguillulina dubia.* Each ovary is made up of a row of egg rudiments which gradually decrease in width as one recedes from the receptaculum seminis. The eggs are cylindrical in shape with rounded ends and measure 73 μ to 79 μ long by 27 μ to 30 μ wide.

Male.—The gonad is single and is outstretched anteriorly as shown in fig. 2. It consists of the four following regions :—(i) *Testis.*—The blind end of this overlies the intestine about half way down the body. It is made up of a number of small cells of fairly uniform size and is a little less than one-third the length of the whole gonad. It gradually increases in width as it joins the next region (ii) *Vescicula seminalis.* —This is a little longer than one-third of the total gonad and is almost as wide as the intestine. Its walls are rather thin and its contents consist of large numbers of small round sperms. (iii) *Vas deferens.*—This is narrower than the previous section and has stout walls. Just in front of the heads of the spicules it tapers steeply to the fourth and final section. (iv) *Ductus ejaculatorius.*—This is the narrow duct connecting the vas deferens with the cloacal aperture. Its final region is obscured by the spicules between which it lies.

The male tail begins to taper a short distance in front of the cloacal aperture behind which it narrows to a conical terminus completely surrounded by the bursa. The latter is voluminous and arises from each

side of the body as a wing a little ventral to the lateral field which here begins to broaden. Figs. 9, 10 and 11 show the tail region in lateral, ventral and dorsal aspect. The free edge of each bursal wing is crenate and the wing itself is striated both on the outside and on the inside. The wing is widest opposite the cloaca. There is a dorsal lobe to the bursa marked off by a small notch on either side. Its free edge is sometimes straight, sometimes faintly indented in the median line, as in fig. 10 and sometimes conical as in fig. 11. There are but few striations on both upper and lower surfaces.



Anguillulina bradys (Steiner & LeHew).

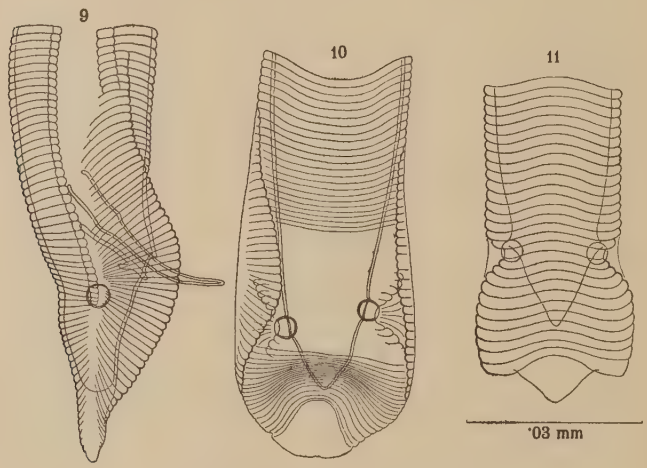
Figs. 6 & 7. Head end and oesophageal region, highly magnified, showing chief anatomical features. Upper scale.

Fig. 8. Male tail in lateral view, highly magnified, showing posterior extent of lateral field, its regular markings in relation to transverse striae of body and lateral caudal papilla. Lower scale.

There is a pair of almost spherical lateral caudal papillae, having the same hyaline appearance and yellowish colour as those on the female tail. They are post-anal in position and, as shown in fig. 10, are not always exactly opposite each other. The outer face of each is at the bottom of a distinct hollow in the side of the bursa towards which the

striae of the body and the bursa can be seen to converge and into which they dip. The hollow, as shown in fig. 9, is in line with the backward extension of the dorsal outer area of the lateral field.

The spicules are of stout build and are slightly arcuate. Each has an expanded head region a little less than one-third as long as the whole spicule. From the head the shaft gradually tapers to a point. The gubernaculum, when seen in lateral view, is shaped as in fig. 9 where it can be seen that there is a short postero-lateral projection not far from the ventral end.



Anguillulina bradys (Steiner & LeHew).

Figs. 9, 10 & 11. Lateral, ventral and dorsal views of male tail respectively, highly magnified, to show structure of bursa, position of lateral caudal papillae, shape of one spicule and gubernaculum. Rectum and ejaculatory duct omitted from fig. 9, spicules, etc., and cloacal aperture from fig. 10.

Systematics.

It becomes necessary to consider the systematic position of the parasite particularly in relation to the genus *Anguillulina*. Daday's (1905) description of *Hoplolaimus tylenchiformis* was based on a single female specimen obtained from river mud in Paraguay. The genus *Hoplolaimus* was not clearly defined until Cobb (1923) amended Daday's description

and gave a diagnosis as follows :—" Coarsely annuled typical tylenchidae with a prominently set off, lobed lip-region composed of several annules and an onchium with more or less lobed basal bulbs. Female gonads paired and opposed, male gonad single. Males with lobed bursa encompassing the tail . . ." He described a new species *H. coronatus*, and drew attention to the presence of a rather distinct, more or less quadrangular network of striations on the lateral fields. In his account of the male of *H. coronatus* he noted the absence of bursal ribs, *i.e.*, lateral caudal papillae, such as are found in many but not all species of *Anguillulina*. He also wrote, " From this amended characterization it seems evident that *Hoplolaimus* Daday (not of other authors) is a rather clearly marked genus." Apart from Daday's somewhat imperfect account of *H. tylenchiformis* and Cobb's description of *H. coronatus* the only other paper on a representative of the genus is that by Steiner and LeHew on *H. bradys*. The latter authors speak of the " very strong spear, the well set off lip region which may have annules or other surface structures. The cuticle has a pronounced annulation and characteristic lateral wings. The bursa of the male is large and envelops the tail end but has no supporting ribs." From these papers by Cobb and Steiner and LeHew it would appear that the principal characters on which *Hoplolaimus* is to be distinguished from *Anguillulina* are as follows :—(i) The lobed or unlobed nature of the lip region with its annulations. (ii) The fact that the anterior faces of the basal swellings of the spear are lobed or indented. (iii) The absence of bursal ribs or lateral caudal papillae. (iv) The presence of a quadrangular network of markings on the lateral fields.

If we consider these four characteristics in especial reference to the parasite dealt with in this paper it can, in the writer's opinion, be shown that they are insufficient to exclude it from the genus *Anguillulina*. The four features may conveniently be taken in the reverse order to which they are set out above.

(iv) *Markings on the lateral fields.*—In the foregoing account of the parasite it has been shown that a quadrangular network of markings is absent from the lateral fields of both sexes. Only here and there are any marks to be found on the inner area of a lateral field namely in the immediate vicinity of the lateral caudal papillae of the female and in the case of one male specimen in which some irregular markings were found on the inner area for a short distance of the neck region. The

markings on the outer areas of the lateral fields are regular in the oesophageal and tail regions where they correspond with the transverse striae. They may be absent from the rest of the body. Similar markings on the outer areas of lateral fields have been found by the writer in the case of *Anguillulina robusta* and *A. multicincta*, both of which have well defined lateral fields.

(iii) *Absence of bursal ribs.*—The large almost spherical lateral caudal papillae of the male correspond exactly in position to the ribs or caudal papillae of many species of *Anguillulina*. The pit-like hollowing in each side of the bursa at the location of each papilla is also present in some species of *Anguillulina*.

(ii) *Indentations on spear bulbs.*—This character also cannot be claimed as quite distinctive of *Hoplolaimus* for the writer has found small lobes or indentations on the anterior faces of the basal swelling of the spear in both *A. robusta* and the closely similar but smaller *A. multicincta*.

(i) *Lip region.*—The structures making up this region in the parasite have been described in some detail. The cuticular head framework is strikingly similar in structure and appearance to that found in such species of *Anguillulina* as *A. pratensis*, *A. robusta*, *A. multicincta* and *A. macrura* in all of which the cuticular loops lying close to the surface of the head produce an appearance of dividing it up into 6 more or less distinct lobes or lip regions. Transverse annulations are also present on the heads of all these species.

It will be apparent from this discussion that, in the writer's view, there are no strong reasons for including the parasite in the genus *Hoplolaimus*. In his opinion it should be classified as belonging to the genus *Anguillulina* and he accordingly names it *Anguillulina bradys* (Steiner and LeHew).

The genus *Hoplolaimus* is thus left with only two species, namely *H. tylenchiformis* Daday, 1905 and *H. coronatus* Cobb, 1923. As already mentioned, the former was inadequately described, consequently our knowledge of the genus must be based on Cobb's species. Whilst this paper was in course of preparation Dr. Gerald Thorne very kindly loaned the writer some mounted specimens of *H. coronatus*. A study of these has made it possible to confirm the fact that lateral caudal papillae are absent from both sexes and that the worms possess peculiar hollow spheroidal organs, one of which lies beneath each lateral field and has

a flattened, ellipsoidal face at the surface of the cuticle. These organs are not un-paired as stated by Cobb but are paired ; one occurring on the right and one on the left side of the body. That on the right is usually pre-equatorial and that on the left usually post-equatorial in position, but in a few cases this arrangement may be exactly reversed. The structure and function of these organs are at present unknown.

In addition to the absence of (i) caudal papillae and (ii) the possession of lateral spheroidal organs, *H. coronatus* presents two further distinguishing features as follows.—(iii) The basal swellings of the buccal spear have distinct sharp points on their anterior faces. (iv) The lateral fields frequently possess cross markings not only on the outer areas but also on the inner area. The above four features serve to distinguish *Hoplolaimus* from *Anguillulina*, to which genus, however, in many general features it is very closely related ; in fact it might very well be considered as a subgenus of *Anguillulina*.

Life-History.

The life-history of the parasite has not been elucidated but judging from the fact that adults and larvae are to be found in large numbers just beneath the skin of affected yams it may be presumed that with the gradual decay of the tissues these would ultimately make their way out into the surrounding soil and so provide a source of infection for other tubers. This is borne out in the brief report by West (1934) where it is stated : " Where crops of yams are grown on the same plots in successive years the disease rapidly builds up to serious proportions." It is evident from the large numbers of larval forms found in decaying yams that, in all probability, more than one generation of the parasite occurs in the parasitized tissues. It has yet to be determined whether any particular larval form constitutes the infective stage or whether the adults themselves can set up fresh infections.

Symptoms.

The disease condition has been termed " Dry Rot of Yams " by West (*l. c.*). Apparently the parasite confines its attentions to the tubers since, up to the present at any rate, no recognisable symptoms of attack have been reported on the aerial regions of the host plants. In this respect

the disease presents some resemblance to that of potatoes attacked by *A. dipsaci* in which also the main seat of attack is in the tubers. The diseased condition is revealed on peeling or lifting the skin of a yam when small areas are found which are at first yellowish but later become brown or black in colour. In these areas the parasites are found and are particularly numerous in older darker lesions just beneath the skin. According to information supplied by Mr. West, the primary lesions caused by the parasite do not penetrate very deep but are confined to the sub-dermal layers. They afford, however, entry for various fungi, among which *Rhizoctonia solani* and *Fusarium oxysporum* are mentioned by West (*l. c.*), to mites and other saprophytic organisms which gradually bring about the destruction of the entire substance of the tuber.

Pathology.

Normal tissues.—In order the better to understand the nature of the pathogenic action of the parasite it is desirable first to give some account of the uninjured tissues. Portions of unaffected yam tuber close to the periphery were selected and sections were cut in a transverse and radial longitudinal direction. After suitable staining these showed the following structures (fig. 12): (1) An outermost dark irregular line in which separate cells are no longer distinguishable, probably consisting of the original epidermis and the immediately sub-epidermal rows of cells. (2) A layer of roundish or polygonal cells very variable in depth; sometimes only two or three and in other places as many as 10 or 12 cells deep. (3) A layer of periderm, again very variable in depth, made up of columns of thin, narrow, brick-like cells. The whole layer has a brownish colour but the outermost 4 or 5 rows are deeper brown than the inner ones. Staining with safranin shows that these cells are suberized; the outer ones more heavily than the inner ones. (4) Within the periderm is a layer of ground tissue the cells of which are somewhat variable in shape and size. They may be rounded or irregularly polygonal in outline close to the periderm and then as we proceed inwards they become narrow and columnar in shape. Intercellular air spaces are small and occur only at the angles of the cells. The latter contain finely granular protoplasm and rounded nuclei. The whole layer, however, is very variable in depth; in some sections it is as much as 15 to 20 cells

deep, in others only 5 or 6 cells deep and the vascular bundles are found lying fairly near to the periderm. Possibly this condition depends on the age of the tuber. (5) Immediately below the columnar cells we find others which are much smaller and irregularly polygonal in shape. They soon become larger in size as we proceed inwards where they are found crowded with starch grains. Amongst these smaller cells the vascular bundles are scattered as small distinct groups of cells.

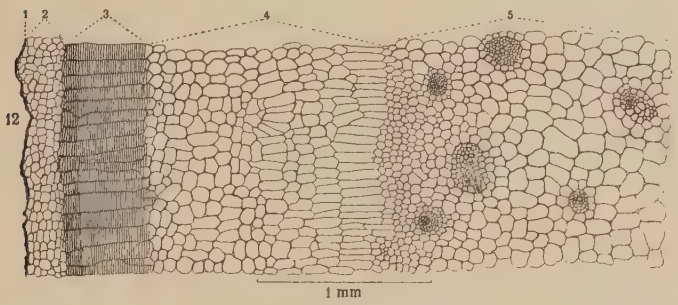


Fig. 12. Portion of transverse section of normal yam tuber to show general anatomy: For description of regions 1, 2, 3, 4 and 5 see text. Cell contents in regions 4 and 5 omitted.

Affected tissues.—The worms confine their activities to the tissues lying within the periderm. In these no cell hypertrophy or cell multiplication is brought about but there is extensive cell destruction. The parasites act intracellularly, not intercellularly, for sections of diseased tissue reveal portions of worms within cell boundaries. Walls of cells are broken down and cell contents are destroyed in some places to such an extent that irregular spaces are formed in which large numbers of the parasite are found. This is particularly noticeable just beneath the periderm. Where cell destruction is abundant the remaining cell walls become coated or impregnated with a brown substance which does not stain with Delafield's haematoxylin. It is also noticeable on the xylem elements of the vascular bundles. The worms also attack the cells lying amongst the vascular bundles and may also be found here and there within cells of the deeper-lying starch-containing layer of the 5th region previously described. They do not seem, however, to occur at a greater

depth than about $\frac{1}{4}$ inch from the outside even in the most heavily invaded sections examined by the writer. In thus confining their destruction to the comparatively surface layers of tissue the parasites resemble somewhat the behaviour of *Anguillulina dipsaci* when parasitizing potato tubers. This parasite also does not penetrate very deeply below the skin. Doubtless in the case of both parasites the general rot of the deeper lying tissues is brought about mainly by the activity of secondary invading saprophytes and saprophages of various kinds. The occurrence of a brown deposit on the walls of cells in the areas attacked is another point of resemblance to the pathogenic action of *A. dipsaci* when parasitizing potato tubers.

Hosts.

Mr. West has informed the writer that in Nigeria the parasite has been found to attack the following species of yam :—*Dioscorea alata* Linn. (water yam), *Dioscorea cayenensis* Lam. (white yam) and *Dioscorea rotundata* Poir. (yellow yam).

Geographical Distribution.

The material from which the parasite was first reported by Steiner came from Jamaica. Steiner and Buhrer recorded its presence in a yam from Porto Rico. In Nigeria, according to West (*l. c.*) it occurs only in the Western Provinces in the farming districts round Ibadan and Illorin.

References.

Cobb, N. A., 1923.—" An Amendation of *Hoplolaimus* Daday, 1905 nec auctores." *Contrib. Sci. Nemat.*, xiii, 363-366.

Daday, E. von, 1905.—" Untersuchungen über die Süsswasser-Mikrofauna Paraguays. *Zoologica Stuttgart*, xliv, 1-374. (*W.L.* 23820.)

Goodey, T., 1932.—" The Genus *Anguillulina* Gerv. & v. Ben., 1859, vel *Tylenchus* Bastian, 1865. *J. Helminth.*, x (2/3), 75-180. (*W.L.* 11224a.)

Steiner, G., 1931.—" A Nematosis of Yams caused by a New Species of *Hoplolaimus*. *Plant. Dis. Rep.*, xv (11), 121.

Steiner, G. & Buhrer, E. M., 1934.—" Observations of interest on Nematode Diseases of Plants. *Ibid.*, xviii (7), 100.

Steiner, G. & Le Hew, R. R., 1933.—" *Hoplolaimus bradys* n. sp. (Tylenchidae, Nematodes), the cause of a Disease of Yam (*Dioscorea* sp.). *Zool. Anz.*, ci (9/10), 260-264. (*W.L.* 23833.)

West, J., 1934.—" Dry Rot of Yams. *Bull. imp. Inst. Lond.*, xxxii (3), 448-450. (*W.L.* 4308.)

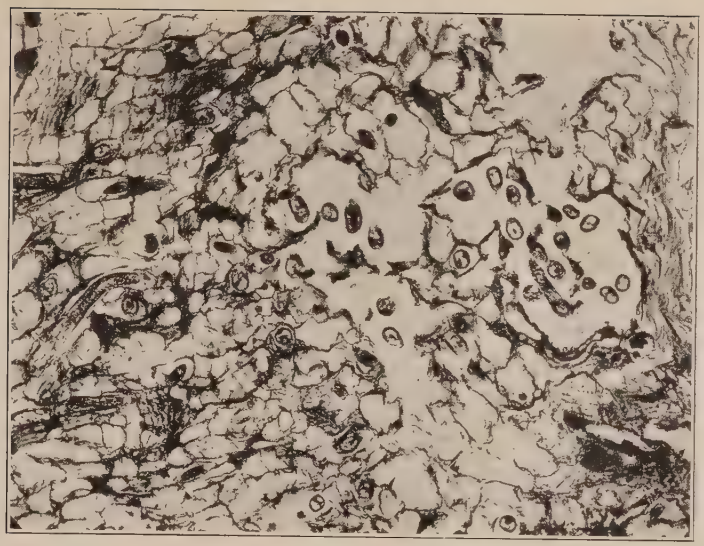


Fig. 13.

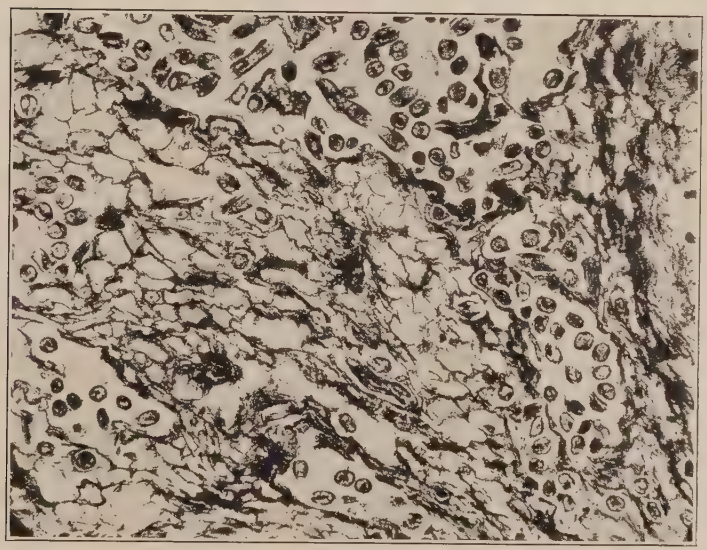


Fig. 14.

Yam Tuber affected by *Anguillulina bradys* (Steiner & LeHew).

Figs. 13 & 14. Sections showing the parasites in irregular cavities formed in broken down tissues close to collapsed periderm on right. x 95.

Journal of Helminthology, Vol. XIII, *No.* 4, *December*, 1935, *pp.* 191-200.

Experiments on the Control of "Potato-Sickness" by the addition of Certain Chemicals to Soil infected with *Heterodera schachtii*.

By Richard H. Hurst, B.Sc., Ph.D., F.I.C.,
and
Marjorie J. Triffitt, D.Sc.

(*From the Institute of Agricultural Parasitology and London School of Hygiene and Tropical Medicine.*)

The problem of affording protection against eelworm invasion to potato plants growing in soil infested with cysts of *Heterodera schachtii* has been investigated with the object in view of adding to the soil a chemical substance which would either (1) have a lethal action on the eggs contained in the cysts, or on hatched larvae during their passage towards the roots of the host ; or (2) tend to neutralise the principle of potato-root excretion which is the stimulating factor concerned in the liberation of larvae from the cysts. The chemicals dealt with in this paper, and which preliminary experiments suggested might possess one or other of the above properties, comprise ferric oxide, ferrous sulphate, ferric chloride, chinosol (8-hydroxy quinoline potassium sulphate) and potassium ethyl xanthate ($KS_2COC_2H_5$)

Laboratory Experiments.

1. *Chemical substances lethal to* H. schachtii.

When hatched larvae were placed in a 2·5 per cent. solution of ferric chloride, they were paralysed after a few hours. Greater dilutions of ferric chloride took a correspondingly longer time to produce the same effect, a solution of 0·5 per cent. requiring several days to produce cessation of movement in all the larvae. Strong solutions of ferrous sulphate were also lethal to the larvae but the action was much less rapid than that of equal concentrations of ferric chloride. Many of the larvae which had been in contact with a dilute solution of chinosol (0·1 per cent.) for 24 hours were still alive, but their movements were very sluggish until they were transferred to distilled water.

Potassium ethyl xanthate was investigated chiefly with a view to the possible lethal effects of the vapours given off by this substance. A

petri-dish containing larvae in distilled water was placed in a larger vessel containing moist xanthate. After three days nearly all the larvae appeared to have been paralysed, whereas in a control experiment without the xanthate, the majority of the larvae remained quite active. It was realised, however, that the quantity and chemical nature of the vapours liberated might be different when the xanthate was mixed with moist soil.

The effects of these substances on the cysts contained in infected soil was then investigated. Air-dried soil (pH 6·3) was passed through a sieve and 50-gram portions placed in glass bottles. These were treated, respectively, with 0·1 gram of anhydrous ferric chloride (a quantity equivalent to 40 cwt. per acre to 9 inches), 0·1 gram of ferrous sulphate (40 cwt. per acre), 0·05 gram of chinosol (20 cwt. per acre) and 0·05 gram of potassium ethyl xanthate (20 cwt. per acre). To the bottle containing xanthate, 10 c.c. of water were added and to each of the other bottles, along with an untreated control, 15 c.c. of water. The mixtures were well-stirred and left for two weeks. After this period the soil was removed from each bottle and the contained cysts were separated by the flotation method. Fifty cysts were selected in each case and placed in a solution of potato-root excretion. The rates of hatching, shown in Table I, indicate that the treatment of the soil had in each case produced some effect upon the cyst contents and that the most promising results had been obtained with potassium ethyl xanthate. In this case only, the solution of root excretion was replaced by a fresh supply at the end of twelve days, but notwithstanding this, only 15 more larvae were liberated

Table I.—Rates of Hatching.

Previous treatment of soil.	No. of cysts used.	Total number of hatched larvae.		
		After 8 days.	After 12 days.	After 20 days.
Control	50	155	280	380
$FeSO_4$	50	60	125	150
Chinosol	50	55	100	110
Fe_2Cl_6	50	50	60	75
K ethyl xanthate ...	50	2	5	20

during the following eight days. In the case of ferric chloride, although the commencement of hatching was not delayed for a significant length of time, the number of larvae liberated was very much smaller than in the case of the control. This would be expected if the ferric chloride had been adsorbed by the soil particles and had therefore not been able to reach all the cysts. The results with chinosol and ferrous sulphate were less satisfactory, a large number of larvae, although definitely smaller than the control, being liberated in each case. It should be noted, however, that the presence of these chemicals in the soil might decrease the power of movement of larvae which had hatched and so tend to prevent their passage towards the roots of the plant.

2. *Chemicals which tend to neutralise the active principle of potato-root excretion.*

The two substances examined in this section were suggested by results obtained during the course of a separate investigation into the properties of the active principle of potato-root excretion. For this purpose, the crude excretion was obtained in a solid state as follows :—

The soil in which potatoes were growing was saturated with distilled water two or three times a week and the solution draining through was collected. This was filtered and evaporated under diminished pressure to about 100 c.c. This was centrifuged to remove insoluble material and treated with successively higher concentrations of alcohol. Various precipitates were formed, collected, and dried with alcohol and ether. The most active fraction was soluble, in the presence of this volume of water, in 75 per cent. alcohol, and although it was still extremely crude, containing over 80 per cent. of ash, it was active in a dilution of 1 part in 500,000 of water.

All the fractions obtained in the above manner contained some active material and it was suspected that this was due to adsorption on the alcohol-precipitates. This was supported by the fact that when a dilute solution of the most active fraction was shaken with vegetable charcoal, the filtrate from this was inactive. In view of this property experiments were carried out to determine whether the addition to root-excretion of a precipitate exhibiting a large surface-area, modified the rate of hatching of *H. schachtii* larvae. The substances investigated, for the purposes of this paper, were iron rust and very dilute ferrous sulphate solution (which by slow atmospheric oxidation deposited a precipitate).

The iron rust was obtained by placing a small rusty nail in root excretion containing cysts and removing it after a few minutes. In both cases hatching was retarded, practically no larvae being liberated during the first week and very few during the second week. After this period, hatching proceeded relatively freely, more particularly in the case of ferrous sulphate.

Small Scale Field Experiments.

The experiments with these five chemical substances were extended to trials on a plot of land at the Institute's Field Station, St. Albans. The plot measured 100 feet by 21 feet and the soil (pH 6·9) was of the heavy loam type containing much clay which is prevalent throughout the district. In 1931 this plot had been infected with *H. schachtii* by the addition of soil from another experimental plot heavily infected with cysts. The strain of eelworm had originally been obtained from Lincolnshire but had been maintained in Hertfordshire since 1925. Potatoes had been grown annually for four successive seasons previous to the experimental treatments described in this communication and the land was markedly and uniformly " potato-sick " throughout.

The plot was divided into 40 rows, the length of each row being 21 feet and the distance between adjacent rows 2 feet 6 inches. " Seed " potatoes were placed at intervals of 1 foot along the rows, this being followed immediately by application of the chemicals to the soil in the vicinity of the tubers while the latter were still exposed. Since the investigations of the effects of chemical treatment were entirely of a preliminary nature, no attempt was made to include a sufficient number of control areas for the purpose of making conclusive comparisons with each treated area. This would hardly have been possible with so small an area of land, unless the number of treatments had been reduced. Moreover, the object of the experiments was to compare the treatments with each other as much as with the control, and it was hoped to receive an indication of whether any of the chemicals was worthy of further systematic study on a larger field-scale. Accordingly, 7 rows at one end and 8 at the other were left untreated. The centre 25 rows, in blocks of 5, received the five treatments.

In place of iron rust, the finely divided precipitated red ferric oxide (Fe_2O_3) was employed. This was applied almost entirely to the sides and bottom of the rows, the " seed " potatoes becoming coated with a fine

layer. Chinosol and potassium ethyl xanthate were placed in the rows between the tubers, care being taken not to place the solid actually in contact with these. The same precaution was taken with ferric chloride and ferrous sulphate, a region of about 1 inch from the " seed " potatoes being left untreated ; in these two treatments, although the soil in the rows received chief consideration, some of the solid was put on the soil between the rows. As soon as the rows had been filled in with soil, the areas which had received ferric chloride and ferrous sulphate were watered. The amounts (expressed as cwt. per acre) of the various chemicals used are recorded in the first column of Table II, but consideration of these quantities should take account of the uneven distributional method adopted.

The potato plants on each treated area developed more rapidly than those on the control areas. Judged by the size and appearance of the plants, those treated with ferric oxide gave the best results. Those which received ferrous sulphate and potassium ethyl xanthate came next, followed by those treated with chinosol and lastly, those treated with ferric chloride. The customary symptoms of eelworm invasion during the early growth of the plant-roots were absent from the ferric oxide and xanthate areas and were definitely less marked in the other areas than in the controls. Since it was desired to compare the yields of potatoes from each row, no attempt was made to remove plants in the early stages of growth for the purpose of making rough comparisons of the numbers of new cysts which had formed on the roots.

Some of the plants were beginning to die down at the end of 18 weeks and the crop was then lifted. The yield from each row was weighed and the total yields from the five rows of each treatment were kept separate. For comparative purposes and to minimise to some extent the effects of " border influence " the untreated area comprising rows number 3 to 7, and that comprising rows number 33 to 37 only were regarded as control areas. The results are given in Table II and shown diagrammatically in Fig. I. The total yield from each treated area was shaken on a riddle of $1\frac{5}{8}$ inch mesh and those too large to pass through were weighed as " ware." From those which passed through, the " seed " potatoes were separated and weighed. The results of these operations are given as weights and percentages in Table III.

TABLE II.—YIELDS.

Treatment.	No. of row.	Yield (lbs.)	Average yield per row (lbs.)
Untreated control.	1	5	8·70
	2	7½	
	3	10	
	4	8¼	
	5	6¾	
	6	10	
	7	8½	
$Fe_2 Cl_6$ (12½ cwt. per acre).	8	8	10·25
	9	11½	
	10	10¼	
	11	9½	
	12	12	
$Fe SO_4$ (15 cwt. per acre).	13	13	12·30
	14	12½	
	15	14¾	
	16	12	
	17	9½	
$Fe_2 O_3$ (6 cwt. per acre).	18	15½	14·35
	19	14½	
	20	14½	
	21	13¾	
	22	13½	
Chinosol (1¼ cwt. per acre).	23	12½	11·80
	24	12½	
	25	10	
	26	12¾	
	27	11¼	

TABLE II—(*Continued.*)

Treatment.	No. of row.	Yield (lbs.)	Average yield per row (lbs.)
K ethyl xanthate (1¼ cwt. per acre).	28	13½	12·05
	29	10¼	
	30	12¾	
	31	10¾	
	32	13	
Untreated control.	33	9½	8·15
	34	10¾	
	35	7½	
	36	7	
	37	6	
	38	8¼	
	39	8½	
	40	8¼	

TABLE III.

Treatment.	Yield (lbs.).			Percentage of yield.		
	"Ware"	"Seed"	"Chats"	"Ware"	"Seed"	"Chats"
Control 1	19¼	5½	18¾	44	13	43
Control 2	16	6	18¾	39	15	46
Fe_2Cl_6	29¾	5¼	16½	58	10	32
$FeSO_4$	33¾	6¾	21	55	11	34
Fe_2O_3	36¾	10¾	24¼	51	15	34
Chinosol	32	9	18	54	15	31
K ethyl xanthate ...	29½	8	22¾	49	13	38

It will be seen from the results in Table II that the highest yield was obtained by ferric oxide treatment. The maintenance of a high yield for each of the five rows is significant, since it is extremely unlikely that the

whole of this particular area happened by chance to be either more fertile on the one hand, or less heavily infested with eelworm on the other. Moreover, there is no reason to suppose that the insoluble ferric oxide would be of much benefit to the plant from a nutritional point of view. It appears probable, therefore, that the action of the ferric oxide was to prevent the penetration of the potato roots by *H. schachtii* larvae, at least in the early stages of development of the plant and before the roots had extended to the regions which had not been treated between the rows. An attempt will be made during the coming season to confirm these conclusions, by observing the effect of ferric oxide treatment on potatoes growing in non-infected soil.

The application of ferric chloride in the solid state to a large area would be rendered very difficult owing to the hygroscopic nature of this substance and the consequent mechanical difficulties in spreading it. Ferrous sulphate is much easier to handle and might be worthy of further consideration. The relatively unsatisfactory results of the various experiments with chinosol, coupled with the high cost of this substance indicate definitely that it has no practical application. From an academic point of view, the results following the use of potassium ethyl xanthate were very promising, but the cost of the treatment would be very high. To illustrate this, mention may be made of pot experiments in which the xanthate was mixed evenly with infected soil in amounts equivalent to 20 and 2 cwt. per acre respectively. After potatoes had grown in these mixtures for 8 weeks, the pots were " turned out " and counts made of cysts on the outer roots. On the plant grown in soil which received 20 cwt. per acre, no cysts were observed but where only 2 cwt. per acre had been applied, over 400 newly formed cysts were counted on the roots of the plant. Intermediate amounts of xanthate dressings have not so far been tried, but the experiment with the smaller quantity was not promising in view of the cost of the chemical. It should be noted, however, that these experiments are not strictly comparable with that on the plot, where the xanthate dressing was confined to the soil along the rows.

It is intended, during the coming season, to carry out a more comprehensive series of experiments with ferric oxide on a larger field scale, and it is hoped to extend the investigations to other crops such as oats, peas and beet growing in soils infected with the strains of *H. schachtii* specialised for these hosts.

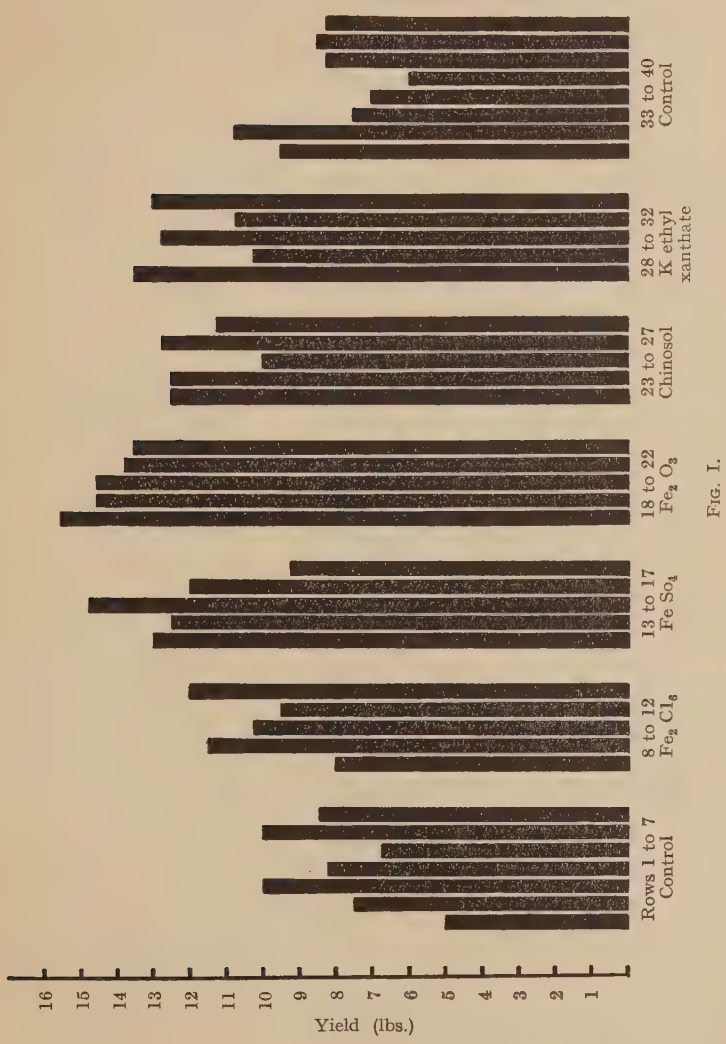

Yield (lbs.)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Rows 1 to 7 Control
8 to 12 $Fe_2 Cl_6$
13 to 17 $Fe So_4$
18 to 22 $Fe_2 O_3$
23 to 27 Chinosol
28 to 32 K ethyl xanthate
33 to 40 Control

FIG. I.

Summary.

1. Laboratory experiments have shown that solutions of ferric chloride, ferrous sulphate and chinosol, in sufficient concentration, have a lethal action on the larvae of *H. schachtii*. The vapours given off by potassium ethyl xanthate were also found to be lethal.

2. Each of these four chemicals was mixed with moist infected soil and the mixture left for a period. When the cysts were separated and placed in a solution of potato-root excretion, the numbers of larvae which hatched were reduced as a result of the chemical treatments, in the order : potassium ethyl xanthate, ferric chloride, chinosol, ferrous sulphate.

3. When ferric oxide was placed in a solution of root excretion containing cysts, the commencement of hatching was delayed.

4. Experiments on a very small field scale were carried out, the chemicals being applied chiefly to the rows in which potatoes were planted. The plants growing on the treated areas did not, in general, show marked symptoms of root-eelworm infection as did those on the control areas.

5. The yields of potatoes from each row are tabulated. The highest yields were obtained following the treatment with ferric oxide.

Acknowledgments.

The authors wish to thank Professor H. Raistrick for his interest in this work and for allowing part of it to be carried out in the Division of Biochemistry, London School of Hygiene and Tropical Medicine.

This work has been largely carried out by aid of a special grant from the Agricultural Research Council.

Journal of Helminthology, Vol. XIII, *No.* 4, *December*, 1935, *pp.* 201-218.

Calcium Cyanamide and other Artificial Fertilisers in the Treatment of Soil infected with *Heterodera schachtii*

By Richard H. Hurst, B.Sc., Ph.D., F.I.C.,
and
Marjorie J. Triffitt, D.Sc.

(*From the Institute of Agricultural Parasitology and London School of Hygiene and Tropical Medicine.*)

During the course of experimental work on the factors governing the escape of larvae from the cysts of *Heterodera schachtii*, a preliminary series of experiments was carried out to determine whether the admixture of artificial fertilisers with infected soil modified in any way the rate of hatching of the larvae. It was thought possible, firstly, that some form of fertiliser might have a lethal effect on the cyst contents or on the larvae during their passage through the soil before they succeeded in entering the roots of the host; secondly, that the fertiliser or its break-down products in soil might neutralise or retard the action of potato-root excretion, which normally stimulates the larvae to escape from the cysts and attracts them towards the roots of the plant; or thirdly, that the larvae might be stimulated to hatch to some extent even in the absence of the host-plant.

Field trials of various chemicals have been carried out by previous workers in different parts of the country. Morgan (1925) used dressings of bleaching powder, sulphur, ground limestone, muriate of potash, sodium chloride, nitrolim (calcium cyanamide) and naphthalene on plots of infected land at Kirton, Lincs. Of these, the napththalene-treated plot showed the best plants, while the potash and sodium chloride treatments yielded fairly good plants, but eelworm was abundant on the roots in every case. Pot experiments carried out simultaneously showed that carbon disulphide and calcium cyanide produced a significant reduction in the number of cysts on the roots. Roebuck (1928) found an improvement in the crop in the year following the use of naphthalene. Edwards (1929) used drained creosote salts, calcium cyanamide, quicklime, calcium cyanide and carbon disulphide and found a marked improvement in the crop on the creosote salts plot, and some improvement where carbon

disulphide, calcium cyanamide and calcium cyanide had been used. Miles (1930) obtained similar results with naphthalene, calcium cyanide and calcium cyanamide. Buckhurst & Fryer (1931) recorded the effects of drained creosote salts applied at 5 and 10 cwt. per acre, bleaching powder at 8 cwt. per acre and naphthalene at 10 cwt. per acre on " potato sick " land in Lincolnshire, Yorkshire and Lancashire. No reduction in the cyst content of the soil was found to follow these treatments although an increase in yield in Lincolnshire occurred on the naphthalene-treated plot. Carroll (1933) as a result of extensive field trials at Rush and the Aran Islands, concluded that creosote salts were ineffective for the control of potato eelworm. He also carried out pot experiments with naphthalene, p- dichlorobenzene, cresylic acid emulsion, carbon disulphide, copper sulphate, potassium permanganate and calcium cyanamide. The results were negative except in the case of calcium cyanamide, which led to better growth of the plant, increase in yield and reduction in the number of cysts on the roots. The amounts of cyanamide used in these experiments is not stated. Davies (1933) reported that a good stand of potatoes was produced in Carnarvonshire, where the land had been treated with calcium cyanamide for the previous three years ; there was, however, heavy infestation with eelworm. Trials with nitro-chalk and sulphate of ammonia showed that these produced as good yields as did calcium cyanamide. Hodson (1934) found that 6 cwt. of calcium cyanamide per acre gave good results, whereas Edwards (1934) found that this amount had no effect. Blenkinsop (1935) obtained a remarkable increase in growth of potatoes in Devonshire, when infected soil had been treated with 12 cwt. of sulphate of potash per acre in addition to 3 cwt. per acre which was present in the customary fertiliser (and which the control received). He presents chemical data, which show that in the control plot the phosphate-potash ratio was abnormally high and that the balance was restored in the plot which received the extra potash dressing. Edwards (1934) also obtained a much greater yield, following the application of 10 cwt. per acre of sulphate of potash. In many other districts, however, negative results have been obtained after treating infected land with heavy potash dressings.

Laboratory Tests on the Effects of Artificial Fertilisers.

The effects of naphthalene and sulphur were first tested. Larvae were found to hatch freely from cysts which had been embedded in moist

sulphur for a week, and to remain alive in contact with sulphur for 48 hours. As no benefit from the application of sulphur had been observed, no further tests were made. Free larvae were also found to be unaffected by remaining in contact with flake naphthalene for three days. As it seemed possible that some decomposition of naphthalene in soil might produce a toxic or lethal substance, 20 grams of naphthalene were thoroughly mixed with 200 grams of soil in a plant-pot. The pot was sunk in soil, out of doors, and kept well-moistened for six weeks. After that period cysts were soaked for a week in the leachings of the pot, and then transferred to dilute potato-root excretion. Newly-hatched larvae of the potato strain were also placed in a petri-dish containing leachings, and kept under observation. It was found that larvae hatched freely from the soaked cysts, and that the larvae placed in the leachings remained alive for as long a time as larvae in distilled water ; some survived for 19 days in each case.

A wider range of tests was then set up which comprised naphthalene and eight artificial fertilisers. The fertilisers chosen were basic slag, superphosphate, muriate of potash, sulphate of potash, kainit, ammonium sulphate, nitrate of soda and calcium cyanamide. To determine whether any of these substances in solution had a stimulant effect on the hatching of *H. schachtii*, one gram of each was well shaken in 50 c.c. of distilled water and left for a week. One hundred cysts were then added to 5 c.c. of the solution in each case, while two controls, using distilled water and potato-root excretion, respectively, were set up at the same time. No larvae escaped except in the control of potato-root excretion. In order to determine whether any neutralisation of potato-root excretion was effected by any of the solutions, 1 c.c. of potato-root excretion was then added to each petri-dish. As a control 1 c.c. of potato-root excretion was added to 5 c.c. of distilled water. Ten days later larvae were present in abundance in all except four cases, these four being solutions of ammonium sulphate, calcium cyanamide, kainit and superphosphate, and of these the two last were very heavily contaminated with fungoid growths. To determine whether the prolonged soaking had had any toxic effect on the embryonated eggs the cysts were removed, washed in distilled water, fungus growths attached to the cysts were removed with a fine brush and each set of cysts was placed separately in dilute potato-root excretion. Except in one instance larvae hatched as freely from the cysts which had

been soaked in fertiliser solutions as from those serving as a control. The cysts which had been soaked in a solution of calcium cyanamide formed the single exception, and from these no larvae were liberated.

As it was realised that the degree of solubility in water of the various fertilisers must differ materially and that the possible decomposition products of the fertilisers in soil might be of more direct application to the eelworm problem than such aqueous solutions, a second series of experiments was set up as follows : Ten six-inch plant pots were filled with soil and a quantity of each of the fertilisers equivalent to the heavy dressing of two tons per acre was weighed out. One fertiliser dressing was then thoroughly mixed into the soil of each of nine pots, the tenth was used as a control and received no dressing. The pots were watered and kept out of doors for 7 days. They were then re-watered and the leachings from each pot were collected and added to a petri-dish containing one hundred cysts of the potato-strain of *H. schachtii*. Three controls were set up, (*a*) leachings of a pot of untreated soil, (*b*) potato-root excretion and (*c*) distilled water. The petri-dishes were examined frequently over a period of 10 days during which time about 1,300 larvae escaped from the cysts in potato-root excretion but no larvae were liberated in the pot-leachings or in distilled water. One c.c. of fresh potato-root excretion was then added to each petri-dish to determine whether any neutralisation of the stimulant substance was effected by the products of the fertilisers present in the leachings. During the next 10 days, hatching took place freely except in the leachings of the calcium cyanamide pot, where only 10 larvae emerged. The cysts were then removed, washed, and placed in fresh root excretion. Again hatching took place freely in all cases except where the cysts had been soaked in calcium cyanamide leachings, and in this instance only 6 larvae were freed, making a total of 16 hatched larvae.

As a result of these two series of experiments it was concluded that basic slag, superphosphate, muriate of potash, sulphate of potash, kainit, naphthalene, ammonium sulphate and nitrate of soda had no lethal or retarding action on the cyst contents of *H. schachtii* either in aqueous solution or in soil over a period of seven days, but that calcium cyanamide when present in sufficient quantity had a toxic and possibly lethal effect on the embryonated eggs and larvae within the cysts. There was, however, no evidence of the root excretions being broken down or neutralised by calcium cyanamide.

Pot Experiments with Artificial Fertilisers.

A preliminary series of pot-culture experiments to investigate the effects of heavy dressings of the fertilisers on plants grown in soil infected with *H. schachtii* was then set up. Dressings at the rate of 10 and 20 cwt. per acre were mixed into soil infected with *H. schachtii*. Naphthalene was omitted from this series but all other fertilisers mentioned above were used. Three control pots of untreated soil were prepared at the same time. A potato " seed " tuber was planted in each pot and allowed to grow for eight weeks. The pots were then removed and the white-stage females visible on the root mass were counted. As the counts were made it became evident that, in general, the amount of root development of the plant was the main factor governing the number of eelworms present on the roots.

Of the three control plants one developed a good root system, the second showed moderate root development but was beginning to die down at the time the counts were made, the third plant failed almost entirely. The eelworm counts were 1,535, 501 and 122 respectively. With the single exception of muriate of potash, the plants grown with fertiliser dressings of 10 cwt. per acre showed comparatively little variation in general condition, root development or eelworm count. All showed evidence of eelworm attack in the condition of the shoots and in general resembled the second of the control plants. The eelworm counts varied from 369 in the soil dressed with basic slag to 769 in the soil dressed with superphosphate. The plant grown in soil with the dressing of 10 cwt. per acre of calcium cyanamide bore 521 white-stage females. Much greater variation was shown in the second series where dressings equivalent to 20 cwt. per acre had been applied. The muriate of potash plant failed completely, and 27 cysts were present on the very ill-developed root system. The plant in soil dressed with nitrate of soda was little better and bore only 58 eelworm cysts. Sulphate of ammonia, basic slag, superphosphate and kainit produced plants of moderate growth and root development, the eelworm counts being 325, 138, 727 and 615 respectively. Conspicuously the best plant was grown in the soil dressed with sulphate of potash, but this bore 1,158 white stage females, *i.e.*, almost as many as the one control plant which made good growth. The plant grown in soil dressed with 20 cwt. per acre of calcium cyanamide showed rather poor growth and root development but bore no evidence of eelworm attack.

From these experiments it is clearly impossible to draw any definite conclusions, but it seems reasonable to suppose that none of the plants which received the lighter dressing of any of the fertilisers derived sufficient benefit to enable them to tolerate eelworm attack. On the other hand the heavy dressing of sulphate of potash may have resulted in an increase of vigour in the host plant which enabled it to withstand a heavy infestation of eelworm. The absence of eelworm cysts on the roots of the plant which received a heavy dressing of calcium cyanamide was significant in the light of the laboratory experiments. Experiments in greater detail were, therefore, carried out with this substance.

Laboratory Experiments on the Toxicity of Calcium Cyanamide.

Commercial calcium cyanamide contains approximately 60% of calcium cyanamide, 12% of carbon and 25% of quicklime. Impurities such as calcium carbide are present in very small amount.

The chemical changes which calcium cyanamide undergoes in various media have been described by Crowther & Richardson (1932). When dissolved in water it decomposes to give an acid salt [$Ca\ (HCN_2)_2$] and calcium hydroxide. Slow hydrolysis of the acid salt leads to the formation of a basic salt [$(CaOH)_2\ CN_2$], along with free cyanamide [H_2CN_2]. Under the influence of the alkaline medium in which it is contained, this free cyanamide undergoes, in the cold, gradual polymerisation to dicyanodiamide [$(H_2\ CN_2)_2$]. The possible toxic effect of a solution of calcium cyanamide on the larvae of *H. schachtii* was examined.

Commercial calcium cyanamide was shaken with distilled water. After remaining for half an hour, a portion of the suspension was filtered. A number of recently hatched larvae were transferred to petri-dishes containing the clear filtrate and others to dishes containing the unfiltered suspension. There was no marked evidence of toxic effect, many of the larvae in each case remaining alive for several days.

Experiments of longer duration were carried out with *H. schachtii* cysts. These were placed in water containing calcium cyanamide and left to soak. The effects produced by varying periods of soaking were as follows :—

(*a*) Some of the cysts were removed after soaking for one week. These were well washed and placed in a dilute solution of potato-root excretion. At the end of one month no hatching had taken place, and the cysts were

transferred to another dish containing fresh root excretion. Hatching now proceeded at a very slow rate, about 100 larvae being liberated during the next two months.

(*b*) Others of the cysts were removed after soaking for two weeks. Hatching, in the presence of renewed supplies of root excretion, was still more delayed than in the previous case. At the end of two months only one larva had been liberated and at the end of three months 20 larvae.

(*c*) The remainder of the cysts were transferred to root excretion after soaking for three weeks. In this case, no hatching took place during a period of three months.

A solution exhibiting greater toxicity was obtained by the following experiment. A small tube containing about 1 gram of calcium cyanamide was tightly plugged with sufficient cotton-wool to fill the air-space above the solid and placed in a petri-dish containing distilled water. Some of the fluid was withdrawn after 24 hours and cysts allowed to soak in it for periods of one and two weeks. In both cases, when the cysts were removed, washed and transferred to root excretion, the rate of hatching was very much retarded during the first month. When placed in contact with a fresh supply of root excretion, however, they hatched freely. The remainder of the fluid was withdrawn after one week and again cysts were allowed to soak in it for periods of one and two weeks. In these cases, no subsequent hatching could be induced by means of root excretion.

When calcium cyanamide is mixed with moist soil, it decomposes rapidly. In very alkaline soils the formation of dicyanodiamide previously described tends to take place, but in normal soils, particularly those showing an acid reaction, the acid cyanamide and free cyanamide which are formed are converted into urea and this in turn into free or combined ammonia. Crowther & Richardson (1932) showed that in normal soils the conversion to ammonia was completed within a few days. The further change to nitrates was slower and less complete. These authors found that the " gaseous products from the decomposition had no perceptible effect on seeds germinating on filter paper over moist soil and calcium cyanamide in closed vessels." These vapours were, however, found to be very toxic to *H. schachtii* larvae, as is shown by the following experiment. About 50 recently hatched larvae were placed in a thin layer

of water contained in a small petri-dish. This was placed in the centre of a large dish (with cover) and surrounded by a small quantity of calcium cyanamide mixed with damp soil. The experiment was carried out in duplicate and in each case all the larvae were killed within three hours. In parallel experiments with moist cyanamide, but in the absence of soil, the larvae remained alive and appeared to have been very little affected.

The rate of decomposition of calcium cyanamide in soil was shown by Crowther & Richardson (1932) to be correlated with the soil moisture content. They found that, for the temperatures of 13·5° and 22·5°C., and for moisture contents varying from 11 to 25 per cent., the cyanamide decomposed more rapidly in the drier soils and at the higher temperatures. On the other hand, when the soil was very dry (5 per cent. moisture) the cyanamide disappeared exceedingly slowly.

Since the gaseous products formed during the decomposition of cyanamide were shown to be lethal to *H. schachtii* larvae, it was important to determine whether the rate of such decomposition, as influenced by varying moisture contents, led to varying degrees of toxicity. Air-dried infected soil was ground in a mortar, passed through a fine sieve and mixed with calcium cyanamide in the proportion of one part by weight to 1,000 of soil. To 100-gram portions, water was added in amounts of 0, 5, 10, 15, 20 and 30 grams, respectively. Each of these was carefully mixed and placed in a large petri-dish, which was then covered. The experiments were carried out in duplicate and the dishes were allowed to remain for five weeks. At the end of this time the cysts were separated from the soil, washed and placed in potato-root excretion. The most rapid liberation of larvae occurred in the case of cysts which had been present in the dishes with no added water. Hatching, however, proceeded quite freely from those which had received 30 and 20 grams of added water per 100 grams of the soil-cyanamide mixture. In the cases of 15 and 10 grams of added water, the commencement of hatching was delayed and comparatively few larvae were eventually liberated. In the case of 5 grams of added water, no larvae hatched during three weeks of observation.

Experiments were also carried out to determine whether any toxicity would remain after the cyanamide had been converted to urea. Infected soil was air-dried, powdered and passed through a fine sieve. 100-gram portions, mixed with varying amounts of urea, were placed in glass jars.

The amounts of urea used were one part in 100, 250, 500 and 1,000 parts of soil, respectively. Sufficient water was added to make the mixtures quite damp and the contents of each jar were well stirred. Control experiments with no urea were conducted at the same time. At the end of two weeks the cysts were separated from the soil, washed well and placed in solutions of potato-root excretion. The cysts from the soil which had contained urea 1 in 100 and 1 in 250 liberated no larvae. Those from the soil with urea 1 in 500 showed a considerable delay in the commencement of hatching and finally only about 30 larvae were liberated. The cysts from the soil with urea 1 in 1,000 showed delayed hatching for the first few days, but later hatched almost as freely as did those of the control experiments.

Further, it was shown by experiment that the decomposition products remaining after the cyanamide had been in contact with *damp* soil for some days were not toxic to *H. schachtii* larvae. A mixture of 1 gram of calcium cyanamide and 100 grams of air-dried soil was moistened with 20 c.c. of water and well-stirred. After one week water was run through the soil mixture and the leachings collected. Cysts which were soaked in this fluid for one week and then transferred to a solution of potato-root excretion, hatched quite freely. In a parallel experiment the same soil-cyanamide mixture was kept dry for one week and leachings then collected. Cysts soaked for one week in this fluid, which contained unchanged cyanamide, could not subsequently be induced to hatch.

Pot Experiments.

For these experiments eelworm-infected soil from Bedfordshire was used. This was of a light sandy type and had a pH of 6·3. Six-inch plant pots were used, this comparatively small size being very suitable for the subsequent operation of turning out the soil with a minimum of breakage, and counting the cysts newly formed on the outer roots.

It was calculated that a mixture by weight of one part of the chemical with 1,000 parts of dry soil would correspond with a field dressing of 1 ton per acre mixed to a depth of 9 inches. On this basis, a duplicate series of pots was prepared, containing the equivalent amounts of 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 cwt. per acre of calcium cyanamide. The object of including very heavy dressings in the series was to observe not only the effect on eelworm cysts but also the response of

the plant to large amounts of calcium and nitrogen. A duplicate series of pots was also prepared with urea in amounts such that the nitrogen contents were equivalent to those of the cyanamide series; *i.e.*, at the rate of 4·5, 6·7, 9, 13·5, 18, 22·5, 27, 31·5, 36, 40·5 and 45 cwt. per acre. The comparison with cyanamide was further extended to smaller series of pot experiments with ammonium sulphate, ammonium nitrate and potassium nitrate, the amounts used being equivalent, on the basis of nitrogen content, to dressings of calcium cyanamide at the rate of 10, 15, 20 and 90 cwt. per acre. In view, also, of the stimulating results on plant growth obtained in the previous single experiment with potassium sulphate, pots were prepared containing this substance in amounts corresponding to 10 and 20 cwt. per acre. A number of controls were set up at the same time. In addition, for all experiments (including the controls), the soil received a dressing at the rate of 5 cwt. per acre of a mixture of one part of potassium sulphate, three of superphosphate and one of ammonium sulphate.

The pots were kept out of doors and watered for 12 days at the end of which time a seed potato was planted in each. The response of the plants to the soil containing heavy dressings of nitrogen was conspicuous in all cases. The cyanamide pots containing less than 20 cwt. per acre produced plants which were not distinguishable from the controls, whereas those containing 30 to 50 cwt. per acre produced an excellent growth of leafage both in amount and colour. Beyond 50 cwt. per acre the improvement was maintained, except possibly in the pots containing 90 and 100 cwt. per acre. Similar results were obtained in the urea series. In the series with ammonium salts and potassium nitrate, the plants which received the heavy dressings of nitrogen were conspicuously the best in appearance. It was observed, too, that the plant responded better to potassium nitrate than to similar amounts of potassium sulphate.

At the end of 10 weeks the pots were " turned out " and counts made of the white cysts showing on the outer roots. The soil did not always leave the pot unbroken so that in these cases accurate counts could not be made. Further, where the root system was very badly developed many portions of the outermost roots were obscured by the soil. The results shown in Tables I–VII are in many cases, therefore, approximations to the actual numbers of cysts present. It is evident, also,

that the development of a good root system by the plant would, *ceteris paribus*, lead to greater stimulation of hatching and cyst formation than in the cases where the roots were less well-developed. For this reason, the condition of the roots in each pot experiment is recorded in the Tables.

TABLE I.—CALCIUM CYANAMIDE.

Amount mixed with soil (Cwt. per acre)	Approximate Nitrogen content (Cwt. per acre)	No. of cysts on outer roots (Pots in duplicate)	Condition of roots
10	2	>100	Very poor and obscured; accurate counts impossible.
		<100	
15	3	>200	Very poor.
		>100	
20	4	9	Fairly good.
		< 50	Fair.
30	6	2	
		1	
40	8	14	
		13	
50	10	0	
		0	
60	12	2	Very good.
		4	
70	14	0	
		0	
80	16	0	
		0	
90	18	0	
		0	
100	20	0	
		0	

TABLE II.—UREA.

Amount mixed with soil (Cwt. per acre)	Approximate Nitrogen content (Cwt. per acre)	No. of cysts on outer roots (Pots in duplicate)	Condition of roots
4·5	2	>100	Very poor; accurate counts impossible.
		<100	
6·7	3	>100	Very poor.
		>100	
9	4	<100	Very poor
		>200	
13·5	6	>100	Poor
		>200	
18	8	<100	Fair.
		<100	
22·5	10	< 50	Fairly good.
		4	Good.
27	12	4	Very good.
		9	
31·5	14	0	
		0	
36	16	1	
		0	
40·5	18	2	
		0	
45	20	0	
		0	

TABLE III.—AMMONIUM SULPHATE.

Amount mixed with soil (Cwt. per acre)	Approximate Nitrogen content (Cwt. per acre)	No. of cysts on outer roots (Pots in triplicate)	Condition of roots
10	2	113	Fair.
		126	
		108	
15	3	104	Fair.
		132	
		90	
20	4	66	Fair.
		75	
		54	
90	18	0	Good.
		8	Fairly good.
		0	Very good.

TABLE IV.—AMMONIUM NITRATE.

Amount mixed with soil (Cwt. per acre)	Approximate Nitrogen content (Cwt. per acre)	No. of cysts on outer roots (Pots in triplicate)	Condition of roots
6	2	107	Fair (cysts immature).
9	3	45	Fair (cysts immature).
		36	
		174	
12	4	44	Fairly good (cysts immature).
		233	
		327	
54	18	47	Good (cysts immature).
		0	
		96	

Table V.—Potassium Nitrate.

Amount mixed with soil (Cwt. per acre)	Approximate Nitrogen content (Cwt. per acre)	No. of cysts on outer roots (Pots in triplicate)	Condition of roots
15	2	>200	Poor.
		346	Good.
		992	Very good.
22·5	3	673	Very good.
		316	Good.
		495	Good.
30	4	52	Good.
		43	Fairly good.
135	18	0	Very good.
		0	Good.
		2 (very small)	Good.

Table VI.—Potassium Sulphate.

Amount mixed with soil (Cwt. per acre)	No. of cysts on outer roots (Pots in duplicate)	Condition of roots
10	>200	Poor.
	>100	
20	105	Good.
	406	

TABLE VII.—CONTROLS.

No. of cysts on outer roots	Condition of roots
<100	Very poor.
<100	Very poor.
<100	Very poor.
<100	Very poor.
201	Poor.
237	Poor.
439	Good.
259	Good.
195	Fair.
208	Fair.

It will be seen from the results that calcium cyanamide and urea have approximately the same retarding effects on cyst formation when the same weights of each are used. Since, however, we are considering urea chiefly as a breakdown product of calcium cyanamide, it is more accurate to base comparisons on the nitrogen content of the fertilisers. This would assume, of course, that the cyanamide had been converted quantitatively to urea. Judged on this basis, calcium cyanamide is seen to be definitely more effective than urea, and therefore confirms the earlier laboratory experience that calcium cyanamide shows toxicity very soon after it is mixed with damp soil and before sufficient time has elapsed for its conversion to urea.

The representatives of possible decomposition products beyond the urea stage, *viz.*, ammonium salts and nitrate, did not, except those with the very heavy dressings, show any retarding action on cyst formation. The plants which received potassium nitrate dressings equivalent to 15 and 22·5 cwt. per acre produced good leafage and comparatively good root systems and the cyst counts were high. The counts in the ammonium sulphate series were lower, but this could be accounted for by the poorer root growth. In the ammonium nitrate series, the cysts formed were,

for the most part, very small and immature. This was the case whether the dressing was equivalent to 6 or to 54 cwt. per acre.

It should be pointed out that the above counts were made after the plants had been growing for 10 weeks, in most cases before full maturity had been reached. Time did not allow us to leave one of each of the series until growth had ceased, when final cyst counts and weights of tubers formed could have been made. It may be of interest, however, to record results which had been obtained in a previous small series of experiments with urea. In this experiment pots were set up in duplicate with dressings of urea at the rate of 2, 20 and 40 cwt. per acre and there were six control pots. The " seed " potatoes used closely resembled each other in size, quality and in the degree of shoot development shown at the time of planting. At the end of 8 weeks the plants were photographed (Plates I & II), and one of each of the urea-treated pots, along with three control pots, were then " turned out." The remaining pots were left for the plants to mature, and were examined after a further 6 weeks. By this time, except in the case of the plant which received 40 cwt. per acre of urea, the cysts were somewhat obscured by the soil and accurate counts

Table VIII.—Urea with Controls.

Treatment or control	Period of growth of plant (weeks)	No. of cysts on outer roots	Wt. of tubers formed (grams)
Urea, 40 cwt. per acre	8	0	Nil
	14	83	95
Urea, 20 cwt. per acre	8	172	Nil
	14	>200	70
Control 1	8	304	23
,, 2	8	174	21
,, 3	8	236	20
,, 4	14	>200	45
,, 5	14	>200	40
,, 6	14	>200	40

could not be made. The plants which received 2 cwt. per acre are not considered, since the one left to mature failed badly; the other was not significantly different from the controls. The results are shown in Table VIII and from these it may be inferred that the cyst counts recorded in Tables I–VII would, in some cases, have been higher if the plants had been allowed to reach full maturity.

Conclusions.

Of the substances dealt with in this paper, namely, sulphur, naphthalene, basic slag, kainit, superphosphate, muriate and sulphate of potash, sulphate of ammonia, nitrate of soda and calcium cyanamide, the last named appears to be the only one which shows promise of application on a field scale to the problem of reducing the viable eelworm content of infected soil. It seems probable, however, that very heavy dressings would be necessary to give satisfactory results. Even in pot experiments, where the cyanamide was intimately mixed with the soil and given any desired amount of moisture, a dressing equivalent to at least one ton per acre to 9 inches was required to limit the production of new cysts to a very small number. This would indicate that some method of mixing the dressing thoroughly and evenly in the soil would be necessary on a field scale, so that the ordinary practice of broadcasting and ploughing in would be unlikely to suffice. The efficiency of any adopted procedure would, of course, be related to the texture of the soil.

The water content of the soil at the time of application of the dressing would influence not only the thoroughness with which the calcium cyanamide could be mixed and incorporated in the soil, but also the degree of toxicity of the dressing on the eelworm population. It is also clear that the soil moisture content would be an important factor in determining the length of time which would have to elapse after applying the cyanamide, before potatoes could safely be planted. In damp soil, or soil which became damp soon after it received the cyanamide, an interval of about 3 weeks would be ample, but in very dry soil and in the continued absence of rain, a much longer interval might conceivably be required for the decomposition of the cyanamide to have reached the stage at which it would no longer be toxic to the potato plant itself.

It may be pointed out that there is no reason to suppose that application of calcium cyanamide, to be effective, should directly precede the planting

of potatoes, for the laboratory tests have shown that the eggs and larvae are killed in the resting stage, *i.e.*, when contained in the cysts. Hence it might prove advantageous to apply the treatment immediately after the potato crop had been lifted, and to follow with some selected rotation of crops before again planting potatoes. The choice of such crops would depend partly on their response to heavy dressings of calcium and nitrogen.

These and other points requiring investigation can only be determined by extended field trials. It is intended to carry out such field-scale investigations in the coming season.

ACKNOWLEDGMENTS.

The authors wish to thank Professor H. Raistrick for his interest in this work and for allowing part of it to be carried out in the Division of Biochemistry, London School of Hygiene and Tropical Medicine.

This work has been largely carried out by aid of a special grant from the Agricultural Research Council.

REFERENCES.

BLENKINSOP, A., 1935.—" Observations on Potato-sick Soils in Devon and Cornwall." *J. Minist. Agric.*, XLI, 1187–1189. (*W.L.* 11365.)

BUCKHURST, A. S. & FRYER, J. C. F., 1931.—" The Problem of ' Potato Sickness.' A Report upon Certain Experiments." *Ann. Appl. Biol.*, XVIII (4), 584–601. (*W.L.* 1025.)

CARROLL, J., 1933.—" A Study of the Potato Eelworm (*Heterodera schachtii*) in the Irish Free State." *J. Helminth*, XI (3), 137–156. (*W.L.* 11224b.)

CROWTHER, E. M. & RICHARDSON, H. L., 1932.—" Studies on Calcium Cyanamide. 1. The decomposition of calcium cyanamide in the soil and its effects on germination, nitrification and soil reaction." *J. agric. Sci.*, XXII, 300–334. (*W.L.* 10966.)

EDWARDS, E. E., 1929.—" The Control of a Serious Potato Trouble." *J. Minist. Agric.*, XXXVI, 234–242. (*W.L.* 11365.)

MILES, H. W., 1930.—" Field Studies on *Heterodera schachtii* Schmidt in Relation to the Pathological Condition known as ' Potato Sickness '." *J. Helminth.*, VIII (2), 103–122. (*W.L.* 11224b.)

Ministry of Agriculture and Fisheries, Monthly Summary of Plant Pests and Diseases, 1932–1934.

MORGAN, D. O., 1925.—" Investigations on Eelworm in Potatoes in South Lincolnshire." *J. Helminth.*, III (5), 185–192. (*W.L.* 11224b.)

ROEBUCK, A., 1928.—" Potato eelworm." *Fruitgrower*, 51–52.

PLATE I (top row).

UREA TREATMENT.

The two pots on the left received urea equivalent to 40 cwts. per acre.
" " centre pots " " " " 20 " " "
" " pots on the right " " " " 2 " " "

PLATE II (bottom row).

UNTREATED CONTROLS.

(To face page 218).

Journal of Helminthology, Vol. XIII, *No.* 4, *December*, 1935, *pp.* 219-222.

On the Thermal Death-point of *Heterodera schachtii*

By Marjorie J. Triffitt, D.Sc.,
and
Richard H. Hurst, B.Sc., Ph.D., F.I.C.

(*From the Institute of Agricultural Parasitology and London School of Hygiene and Tropical Medicine.*)

It is a recognised fact that the potato-strain of the eelworm *Heterodera schachtii* is distributed from district to district by the agency of cysts embedded in soil adherent to seed tubers. The need for some method of freeing seed tubers from viable eelworm, and the extent to which infection may be carried by seed grown in eelworm infested land, are strikingly illustrated by observations carried out by the staff of the Ormskirk Potato Testing Station, which, by permission of the Director of the National Institute of Agricultural Botany, are quoted here.

Seed tubers grown in soil known to be infected with *H. schachtii* were saved with the purpose of planting on clean land in the following year. They were lifted during dry weather and saved with extreme care, all soil being shaken off as far as possible, and every precaution being taken to avoid soil adhering to the potatoes. The boxes containing these tubers were stacked apart from all other potatoes in the sprouting loft. Before planting it was decided to test the efficiency of the precautions which had been taken. Three boxes were selected at random, the remaining soil particles were brushed off the tubers on to white paper and the soil from the boxes was collected in a similar way. Cyst counts from the soil so collected were made with the following results :—

(*a*)	Soil from 32 tubers	35	cysts.
	,, ,, box	426	,,
(*b*)	Soil from 32 tubers	14	,,
	,, ,, box	392	,,
(*c*)	Soil from 32 tubers	20	,,
	,, ,, box	383	,,

In order to insure that infection was not transmitted to clean land it was found necessary to scrub the tubers in water with a soft brush.

Since hot-water treatment has proved an efficient and economic method of controlling *Anguillulina dipsaci* in bulbs it was thought that a modification of this treatment might prove to be at least a possible method of sterilising seed potatoes against the transmission of *H. schachtii*. It has been recorded in a recent publication that the resting stage of *H. schachtii*, *i.e.*, the embryonated eggs contained in the brown cyst, can withstand high temperatures for considerable periods when the heat is applied in the form of dry, heated air. The first step towards devising a sterilisation treatment by the use of hot water has been to determine the temperatures and times of exposure lethal to the nematode. *Anguillulina dipsaci* can be controlled by exposure to the comparatively low temperature of 110°F. This was thought to give some indication of the temperature range likely to prove lethal to *H. schachtii*, and 110°F. was accordingly used for the first series of tests.

The experimental methods adopted were as follows. A quantity of cysts of the potato-strain were isolated from soil and these were placed in batches of 100 in small glass tubes, the mouths of the tubes being covered with gauze. A thermostatically controlled water-bath was then heated to the desired temperature, the tubes were filled with the heated water by means of a pipette sufficiently fine to pass through the gauze and were then dropped into the water-bath and removed after the desired period of time had elapsed. After treatment the cysts were removed from the tubes and placed in small petri-dishes containing a solution of potato-root excretion which was removed and renewed from time to time while the cysts were kept under observation. Cysts which had not been subjected to hot-water treatment were used as controls to each experiment.

From observations thus carried out the following results were recorded. A temperature of 110°F. maintained for periods up to 1 hour failed to retard in the larvae the power of hatching from the cysts. A period of 30 minutes at a temperature of 115°F. was equally without effect, but 1 hour at this temperature caused a definite delay in hatching, for whereas untreated larvae were hatching freely after 1 week's immersion in the solution of root excretion, very few larvae hatched from the treated cysts in the first 3 weeks, but 3 months after treatment, following repeated applications of stimulant, they emerged very freely. Treatment at 116°F. for 30 minutes caused a delay of about a month in hatching and comparatively few larvae hatched during the four months that the

cysts were kept under observation. No hatching whatever took place from cysts maintained at 116°F. for 45 and 60 minutes. Cysts treated for 5 minutes at 118°F. and 120°F. appeared to be unaffected whereas 10 minutes treatment produced a retardation effect and 15 minutes proved lethal to the majority of the larvae. From cysts treated at 120°F. for 15 minutes only 20 larvae escaped in four months. Thirty minutes exposure to temperatures of 118°F. and 120°F. killed the entire cyst contents, and 5 minutes exposure to 130°F. also proved lethal.

Briefly it may be said that a temperature of 116°F. sustained for a period of not less than 45 minutes kills the entire cyst contents. Higher temperatures kill in shorter periods of time, ranging from 30 minutes at 118°F. to 5 minutes at 130°F. Shorter exposures to these temperatures may result in a retardation of development which may be prolonged for several weeks.

It has been shown that potatoes are uninjured by hot-water treatment at 110°F. for periods of up to an hour and that they may indeed derive some benefit from an immersion of 20 minutes duration at this temperature. In view, however, of the fact that higher temperatures are necessary to kill *H. schachtii* than prove lethal to the resting stage of *A. dipsaci*, extended trials must be carried out to determine the resistance of the seed tubers to this form of treatment before its commercial application can be considered.

Since the retardation of development shown by the nematode in certain instances is sufficient to render useless the normal application of the indicator-plant method of testing viability, it seems advisable to publish the results of these laboratory experiments although no data are yet available regarding the effects of such treatments on the growth of the tubers.

The use of the indicator-plant method, modified and adapted to the retardation periods indicated by the laboratory tests cited above, remains the only reliable test of the survival of infective larvae following heat treatments, for it is yet uncertain whether treatments which produce a marked retardation effect on the larvae may not also reduce their vitality sufficiently to prevent a normal completion of the life-cycle.

A comparison of the results obtained by applying dry-heat and hot-water treatment to cysts of the potato-strain of *H. schachtii*, with results obtained by Fuchs on heating cysts of the beet-strain in dry or moist soil, points to a possible conclusion of some interest.

Fuchs found that the application of heat, when prolonged, tended to produce a retardation of development; that the cyst contents were destroyed by exposure for 48 hours to 50°C. (122°F.), for 24 hours to 55°C. (131°F.), and for 6 hours to 60°C. (140°F.). He concluded as a result of these tests that it mattered little whether the soil in which the cysts were contained was dry or moist, stating however, that the cysts appeared better able to withstand higher temperatures when they had not absorbed too much moisture, *i.e.*, when they were contained in dry soil. In a second series of tests where dry soil only was used, no living larvae were found after 10 minutes exposure to 60°C. (140°F.), after 5 minutes exposure to 62°C. (143·6°F.) or after 1 minute's exposure to 63°C. (145·4°F.).

As Fuchs used the indicator-plant method of determining whether larvae survived the treatments, and these were examined three weeks after sowing (*Synapsis alba* was the species selected to serve as indicator), it is possible that in some cases retardation of development of the nematode induced by the treatment may have obscured the results.

Nevertheless it appears that in a dry state *H. schachtii* can withstand exposure to temperatures which prove lethal when the cysts are contained in soil, and the results recorded by Fuchs suggest that even "dry" soil may contain sufficient moisture to influence the reaction of the nematode to heat. On the other hand, the subjection of cysts to heat under conditions of excessive moisture, such as obtain in the hot-water treatments described above, proves lethal at lower temperatures and with shorter exposures than occurs when only a moderate amount of moisture is present. Hence it would seem that the degree of moisture by which the cysts are surrounded during the application of heat is an important factor in determining the reactions of the nematode.

References.

Anon., 1934.—"The Spread of Potato Eelworm in Consignments of Seed Potatoes." *10th Ann. Rep. Dep. Pl. Path. Seale-Hayne agric. Coll.*, 5–6. (*W.L.* 18109a.)

Fuchs, O., 1911.—"Beiträge zur Biologie der Rübennematoden *Heterodera schachtii*." *Z. landw. VerWes. Öst.*, xiv, 923–949. (*W.L.* 23488.)

Triffitt, M. J., 1935.—"On the origin of strains of *Heterodera schachtii* occurring in Britain, with special reference to the Beet-strain." *J. Helminth.*, xiii (3), 149–158. (*W.L.* 11224b.)

Journal of Helminthology, Vol. XIII, *No.* 4, *December*, 1935, *pp.* 223-228.

Brevibucca saprophaga gen et sp. nov., a nematode from a rotting lily bulb-scale.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

Mr. L. Ogilvie, Mycologist, of the Research Station, Long Ashton, Bristol, recently sent the writer some bulbs of *Lilium longiflorum* Thunb., var. *takesima*, requesting an opinion on the eelworms occurring in the brownish tips of some of the scales. These turned out to be chiefly a species of *Rhabditis* and *Diplogaster nudicapitatus* Steiner. There was one thoroughly decomposed bulb scale, dark brown in colour and of a soft consistency throughout which, on teasing in water, was found to contain several insect larvae, mites and numerous nematodes including specimens of *Diplogaster longicauda* and *D. striatus*. In addition, about 25 adults of each sex of a new genus of nematode were found which, though somewhat resembling Rhabditis in buccal and oesophageal characters, differed from that genus in certain important features the most outstanding of which was the short buccal cavity. On this character the generic name has been based and because the worms were found in a thoroughly decayed organic medium a specific name has been chosen which denotes their probable feeding habits. Mr. Ogilvie has informed the writer that the bulbs were from stocks imported from Japan. Such bulbs arrive in this country in cases packed with Japanese soil but as the bulbs examined had already been planted in English soil it is impossible to determine from the material available whether the newly discovered nematodes were originally inhabitants of Japanese or English soil.

Brevibucca new genus.

Generic diagnosis. Rhabditidae : Mouth surrounded by six low lips each bearing at least one small papilla. Opening of amphid slit-like on lateral lips at about level of lip base. Buccal cavity a little longer than wide ; sides very slightly convex in optical section. Oesophagus Rhabditis-like with corpus merging into median bulb ; isthmus leading to posterior bulb containing valve apparatus.

Female : vulva in posterior part of body just in front of anus ; gonad single, prodelphic, ovary reflexed and reaching far back in body. A pair of ovoid glands present, one on either side of end of uterus.

Male : gonad single, testis outstretched. Spicules paired, right spicule smaller than left, gubernaculum large. Numerous caudal papillae.

Type species *Brevibucca saprophaga* n. sp.

Brevibucca is closely related to *Rhabditis* in the shape of the oesophagus, the posterior bulb of which contains a valve apparatus. It differs from *Rhabditis*, however, in the short buccal cavity, in the presence of a pair of ovoid uterine glands, in the shape of the male tail which is more like that of certain species of *Diplogaster* and in the fact that the spicules are dissimilar in shape and size.

Morphology.

Dimensions. *Female* : length, 0·99 mm. to 1·13 mm., α=14·4 – 16·2, β=4·3–4·56, γ=6 – 8, V=77·54% to 81·6%. *Male* : length, 0·79 mm. to 0·98 mm., α=17·3 – 21, β=3·9 – 4·55, γ=8 – 9·6, spicules, right, 32 – 33 μ, left, 45 – 48 μ, gubernaculum, 30 – 32 μ.

The body in both sexes is of rather stout build and tapers in the oesophageal region towards the head end. Posteriorly the female body tapers rapidly behind the anus to a slender, sharply pointed tail. There is a small papilla on each side of the female tail at about one-third of its length from the anus. In the male the body becomes roundly conical behind and ends in a distinct, slender, sharply pointed tail. The cuticle carries fine longitudinal striations bearing very narrow transverse striae which appear to encircle the body completely. Apparently lateral fields are absent, for even under high magnification, it has not been possible to detect their presence by carefully focusing on the surface of the body.

The mouth is surrounded by 6 low but slightly rounded lips each of which, when viewed laterally, is seen to carry a small papilla. *En face* views of the head end have not been obtained. The amphid opening is a small transverse slit on each lateral lip at about the level of the base of the lips. The buccal cavity or protostom is from 10 to 12 μ deep and has an internal diameter of 6 to 7 μ. The protorhabdions composing its walls are not perfectly plain, straight rods such as are found in species of Rhabditis but are shaped as in fig. 5, where it can be seen that they are

narrow in front and gradually increase in width posteriorly. Each is very slightly concavo-convex in outline. The base of each is expanded and indented a little behind where it articulates with the lateral elements of

Brevibucca saprophaga gen. et sp. n.

Figs. 1, 2 & 3. Adults under low magnification to show general shape and structure. Figs. 1 & 2 are seen from right and left sides respectively so as to show the arrangement of the female gonad.

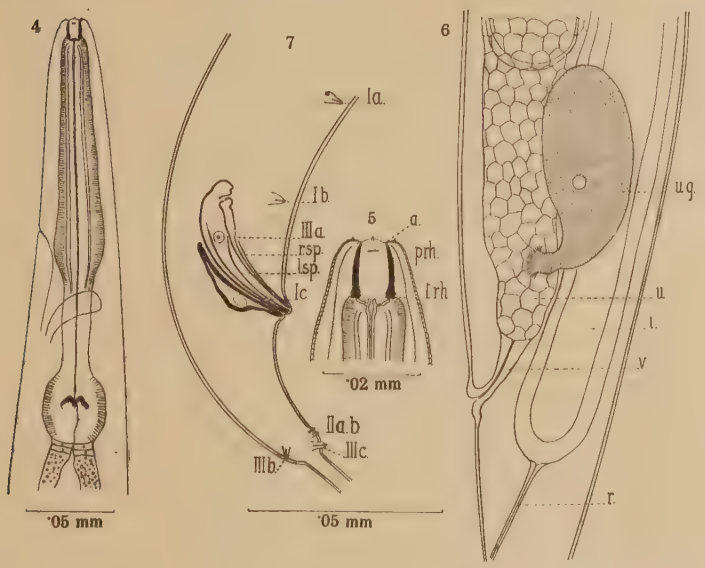
the telostom. The latter is a shallow structure at the anterior end of the oesophagus and on either side there is an irregularly shaped telorhabdion on which the base of each protorhabdion rests but from which it is separated by a narrow space. The telorhabdions appear to be continuous with the cuticular covering of the anterior end of the oesophagus which, at the centre, is raised into a small prominence on either side of the entrance to the lumen.

The oesophagus itself is shaped very much as in species of Rhabditis and is composed of a corpus which increases in width posteriorly and blends with the median bulb. In the corpus the muscular walls are supported by cuticular rods whose front ends curve inwards. The median bulb tapers behind to the isthmus which swells a little in the middle and has a length a little less than one-quarter the total length of the oesophagus. The posterior oesophageal bulb is well developed and towards its centre carries a valve apparatus the front faces of which are finely serrated. The nerve ring crosses the isthmus about the middle of its extent. The excretory pore lies well in advance of the nerve ring at the level of or slightly in advance of the median oesophageal bulb. The intestine extends throughout the body and is connected with the anus by a distinct rectum.

Female. The vulva is placed well back on the ventral side of the body. Its lips are rounded but in life they are not protuberant. However, in specimens killed by heat it is frequently found that the vulva stands out on a rounded prominence. The vagina leads inwards and forwards and its walls are slightly thickened. A noticeable feature in this region of the body is a pair of ovoid glands which are situated one on either side of the end part of the uterus. Their contents are sometimes rather dense and very finely granular and each possesses a single round nucleus. Even when they are empty the outline of each gland can be distinguished.

The lower end of each gland narrows to form a short stalk which is connected laterally with the wall of the uterus a little in advance of the union of uterus and vagina. At the junction, the gland stalk seems to be expanded outwards as shown in fig. 6. The function of these glands is problematical but possibly their contents are discharged during egg-laying. The uterus extends forwards on the left side of the body ventral to the intestine. From one to five eggs at a time have been found within it. At about two-thirds of its length from the vulva it expands to form a

receptaculum seminis in which numerous rounded sperms have been seen. In front of this the oviduct may contain one or two unfertilized ova and then the organ becomes reflexed on itself. The ovary extends backwards on the right side of the body and, gradually decreasing in width towards the tip, reaches in some cases to the level of the uterine glands.



Brevibucca saprophaga gen. et sp. n.

Fig. 4. Oesophageal region in lateral view.

„ 5. Head end highly magnified, showing structure of protostom and telostom.

„ 6. Vulval region seen from left side, highly magnified, showing disposition of one ovoid uterine gland and its attachment to wall of uterus.

„ 7. Posterior end of male seen from right side, highly magnified. a, amphid opening; i, intestine; l.sp. left spicule; prh, protorhabdion; r, rectum; r.sp., right spicule; trh, telorhabdion; u, uterus; u.g., uterine gland; v, vagina.

Male. The posterior part of the male body is always more or less ventrally flexed and this is accentuated in specimens killed by heat, as shown in fig. 3. It can be seen that the body tapers from a little in

front of the cloacal opening to a rounded conical terminus which carries a narrow tail tapering to a sharp point. The gonad is single and stretches forwards in the body. It is made up of the usual regions. The testis was found to be double in one specimen examined but in all the others was single. The anterior end is not as a rule reflexed but in the case just mentioned with two testes, one of them was folded on itself. The vesicula seminalis follows the testis and is somewhat wider than it. It occupies about one-third the length of the whole gonad and leads into the vas deferens whose walls seems to be highly vacuolate. The end of the vas deferens is connected with the cloacal aperture by the narrow ductus ejaculatorius.

The spicules and gubernaculum are comparatively large and of a light brown colour. They are shaped as shown in fig. 7, where it can be seen that they are dissimilar in size and shape. The right spicule is straighter, smaller and slenderer than the left. Its anterior third is somewhat expanded and is cephalated by constriction. The shaft tapers to a point. The left arcuate spicule has a rather prominent rounded head which is separated from the widest part of the shaft by a constriction. The head is notched or indented on its anterior face. Behind the neck constriction the body of the spicule soon reaches its greatest width and then tapers gradually to the tip. The gubernaculum is also arcuate and follows the curvature of the left spicule closely. It increases in thickness from behind and is widest at about two-thirds of its length from its ventral end as shown in fig. 7.

There are 8 pairs of caudal papillae, 6 pairs of which are large and distinct. They are distributed on the posterior part of the body in somewhat the same arrangement as the male caudal papillae of *Diplogaster longicauda* and *Cylindrogaster longistoma*. There are three pairs of ventro-lateral papillae, Ia, b, and c. Ia and Ib are pre-anal in position whilst Ic is ad-anal. On the ventral side of the body, just before it gives rise to the narrow tail process, there are two pairs of very small papillae IIa and b. The lateral papillae are made up of three pairs, IIIa, b and c. IIIa is pre-anal, lying in a true lateral position between the levels of Ib and Ic. The other two of this group are at the end of the body in the region of IIa and IIb. One of them, IIIb, is postero-laterally directed and the other, IIIc, is ventro-laterally directed.

Index to Volume XIII.

	PAGE
Acquired immunity to *Nematodirus*	53
Age resistance, absent in *Nematodirus*	52
Anguillulina bradys in Yams	173
dipsaci on potatoes	91
Aphelenchoides hodsoni n. sp., in Narcissi	167
Artificial fertilisers and *Heterodera schachtii*	201
Birds, helminths of British	146
Brevibucca saprophaga g. et sp. n., in Lily	223
Caterpillars, *Rhabditis coarctata* on	13
Chickens, infected with gapeworms from Starlings	1
Complement Fixation, in *Schistosoma japonicum*	25
Cylindrogaster curzii n. sp.	19
Dry-rot in Yams	187
Eisenia foetida, as intermediate host of Gapeworm	1
Flea-beetles, incidence of *Howardula phyllotretae* in	163
Gapeworm disease, carbon tetrachloride in	6
garlic oil in	4
tracheal nodules in	9
transmission from Starlings to Chickens ...	1
treatment of	3
Heterodera schachtii and "microcysts" in soil	59
bionomics of	77
bodies resembling cysts of	59
effect of artificial fertilisers on	201
heat sterilisation of infected soil	219
in soil, chemical control of	191
oat-strain in Britain	129
origin of British-strain of	149
thermal death-point of	219

PAGE

Howardula phyllotretae in flea-beetles 163
Immunity to superinfection with *Nematodirus* spp. 54
Lilium longiflorum affected by *Brevibucca saprophaga* 223
" Microcysts " and *Heterodera schachtii* cysts in soil 59
Narcissus, affected by *Aphelenchoides hodsoni* n. sp. 167
Necator suillus, differentiation from *N. americanus* 67
Nematodirus spp. in New Zealand sheep 41
Oats, *Heterodera schachtii* strain in 129
Oncomelania hupensis, cercarial antigen from 25
Partridges, helminths of 139
Pheasants, tracheal nodules from gapeworm 9
Potato-sickness, chemical control of 191
Potatoes, *Anguillulina dipsaci* infection of 91
Heterodera schachtii infection of 77
Rhabditis coarctata on caterpillars 13
Schistosoma japonicum, Complement-Fixation in 25
Sheep, *Nematodirus* spp. in New Zealand in 41
Starlings, chicken gapeworm transmitted to 1
Syngamus trachea, experimental transmission of 1
Tan liquor, vinegar eelworm in 159
Thermal death-point of *Heterodera schachtii* 219
Trichostrongylidae, induction of second larval ecdysis 103
larvae behaviour in sterile media 115
Turbatrix aceti in tan liquor 159
Vinegar eelworm in tan liquor 159
Yams, infected by *Anguillulina bradys* 173

Index of Authors.

	PAGE
ANDREWS, M. N.	25
BUCKLEY, J. J. C.	67
CARROLL, J., and MCMAHON, E.	77
CLAPHAM, P. A.	1, 3, 9, 139
EDWARDS, E. E.	129
GOODEY, T.	19, 91, 167, 173, 223
HURST, R. H., and TRIFFITT, M. J.	191, 201
LAPAGE, G.	103, 115
OLDHAM, J. N.	13, 163
PETERS, B. G.	159
TETLEY, J. H.	41
TRIFFITT, M. J.	59, 149
TRIFFITT, M. J. and HURST, R. H.	219

New Names in Volume XIII.

NEW GENERA.

	PAGE
BREVIBUCCA Goodey, 1935	223

NEW SPECIES.

APHELENCHOIDES HODSONI Goodey, 1935	167
BREVIBUCCA SAPROPHAGA Goodey, 1935	223
CYLINDROGASTER CURZII Goodey, 1935	19

MADE AND PRINTED IN ENGLAND